Br. J. Pharmac. (1973), 49, 29–36.

The role of the substantia nigra in the locomotor stimulant action of amphetamine

B. COSTALL AND R. J. NAYLOR

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP

Summary

1. The electrolytic brain lesion technique was used to evaluate the role of the substantia nigra in the mediation of the locomotor stimulant effect of (+)-amphetamine in the rat. The effect upon the results of variations in strain and basal activity levels of the rats was assessed.

2. In the immediate postoperative phase the lesioned animals developed spontaneous stereotyped behaviour patterns which were more intense in rats of initially high basal activity. Activity was depressed at this stage and only that of the low activity animals was stimulated by amphetamine.

3. After the 2nd postoperative day rats with lesions of the substantia nigra developed hyperactivity, but this was only maintained in rats initially of low basal activity. During this stage the locomotor stimulant effect of amphetamine (1·0 mg/kg) was apparent and after the 9th postoperative day animals generally displayed an increased sensitivity to the drug effect.

4. The involvement of the substantia nigra with the control of locomotor activity is indicated but its integrity would not appear essential for the mediation of the locomotor stimulant effects of amphetamine.

Introduction

Amphetamine has been shown to cause a release of dopamine from neuronal terminals and Carlsson (1970) suggested that this effect may mediate the stimulation of locomotor activity which results from amphetamine treatment. The neostriatum would appear to be a suitable anatomical substrate for the amphetamine action since Costa, Groppetti & Naimzada (1972) have shown an association between an increase in locomotor activity and the acceleration of striatal dopamine turnover induced by (+)-amphetamine. Since neostriatal dopamine turnover would appear to be controlled via the substantia nigra and nigrostriatal pathway (Faull & Laverty, 1969), this system may be important for mediation of the amphetamine effect. The absence of a normal running response to (±)-amphetamine after bilateral electrolytic coagulation of the substantia nigra (Simpson & Iversen, 1971) would support this hypothesis but Creese & Iversen (1972) have subsequently failed to modify the locomotor stimulant action of (+)-amphetamine when using an apparently more specific technique for lesion of the substantia nigra. Further, the relevance of the striatum itself has been questioned since extensive lesion of this area failed to modify the amphetamine effect (Naylor & Olley, 1972).

Our previous studies have shown that the locomotor activity responses of rats to drugs are critically dependent upon the basal level of activity of the animals

used (Costall, Naylor & Olley, 1972a) and some of the inconsistencies in previous reports may arise from lack of animal selection. The present studies were, therefore, designed to assess the importance of possible variations in amphetamine effects arising from differences both in basal activity levels and rat strain to an evaluation of the role of the substantia nigra in the mediation of the locomotor stimulant effect of amphetamine.

Methods

Animals and housing

Two strains of Sprague-Dawley male rats, C.F.E. and C.S.E. were used, weighing 125 to 150 g at the commencement of training in the activity wheels and 250 to 300 g at the time of operation. Animals were normally housed in groups of 5 at a temperature of $21 \pm 1°$ C. The housing room was maintained in darkness between 08·00 and 20·00 h and light between 20·00 and 08·00 h.

Measurement of activity

Activity was recorded with activity wheels, 300 mm diameter and 140 mm wide, banked in groups of 10 in a sound-proofed room maintained in darkness at a temperature of $21 \pm 1°$ C. Animals were placed in the wheels for a 6 h period between 09·00 and 18·00 h, the number of wheel revolutions being recorded electromechanically and noted every 15 to 30 minutes. After 6 training sessions animals of both strains were found to divide naturally into low (10 to 100 rev/h) and high (100 to 200 rev/h) activity rats. Rats exhibiting an activity falling outside these divisions were rejected. Correct division of the rats was checked daily (3 to 6 day) until they had attained operative weight.

Lesion procedure

Lesions were induced bilaterally by the method of electrolytic coagulation. Rats were anaesthetized with chloral hydrate, 300 mg/kg, i.p., and a stainless steel electrode (diameter: 0·64 mm), insulated except at the tip, was inserted to the anterior, vertical and lateral co-ordinates of 2·2, $-1·7$, $\pm 2·0$ (De Groot, 1959) with a Kopf stereotaxic instrument. Coagulation was caused by passing 0·5 mA for 10 s, the stereotaxic frame serving as the inert electrode. The bilateral lesions were performed on the same occasion.

Sham-operated animals were treated in exactly the same way except that no current was passed after insertion of the electrode to the appropriate depth (anterior 2·2, vertical $-1·2$ and lateral $\pm 2·0$). This induced an electrode track without damage to the area under study thus allowing the evaluation of any artifact arising from unavoidable damage to overlying structures. Control animals were not subjected to any operative procedure.

Upon completion of the experiments, the positions of the lesions were confirmed by the histological techniques previously outlined by Costall & Naylor (1973).

Experimental design

In initial experiments to establish the dose-dependent nature of amphetamine locomotor stimulation, 5 doses of amphetamine (0·0, 0·5, 1·0, 1·5 and 2·0 mg/kg, i.p.) were administered to 25 rats according to a 5×5 Latin square.

In experiments to determine the effects of substantia nigra lesions upon the amphetamine effect groups of 30 animals were used which were divided into 3 sub-groups of 10 operated, 10 sham-operated and 10 control animals. The sub-groups were placed alternately in 3 banks of 10 wheels to minimize wheel variation. Within each sub-group 5 animals received amphetamine, 1 mg/kg, i.p. (selected as causing a reliable stimulation of locomotor activity but no stereotyped behaviour), and 5 received solvent injection immediately before placing in the wheels. The rats were selected for injection and placed in the wheels at random.

Rats were used 1, 2, 3, 4, 7, 9, 11 days and 3 months after induction of the lesions. Each experimental block was repeated twice for both high and low activity C.S.E. and C.F.E. rats, giving initially 15 determinations for amphetamine and solvent effects. However, mortalities occurred within the operated group from day 4 onwards such that the total number of determinations for amphetamine and solvent was reduced to a minimum of 6 or 9 on day 11 and 4 or 8 at 3 months for low and high activity animals respectively.

The significance of the results was assessed using an appropriate form of Student's *t* test.

Drugs

(+)-Amphetamine sulphate (Sigma) was dissolved in 0·9% w/v NaCl solution (saline) and administered by the intraperitoneal route in a volume of 1 ml/kg.

Results

Dose-dependent nature of amphetamine-induced stimulation of locomotor activity

Amphetamine, in doses greater than 0·5 mg/kg, was found to increase the locomotor activity of rats in a dose-dependent manner (Figure 1). Some animals treated with 1·5 mg/kg amphetamine exhibited a very mild stereotyped behaviour

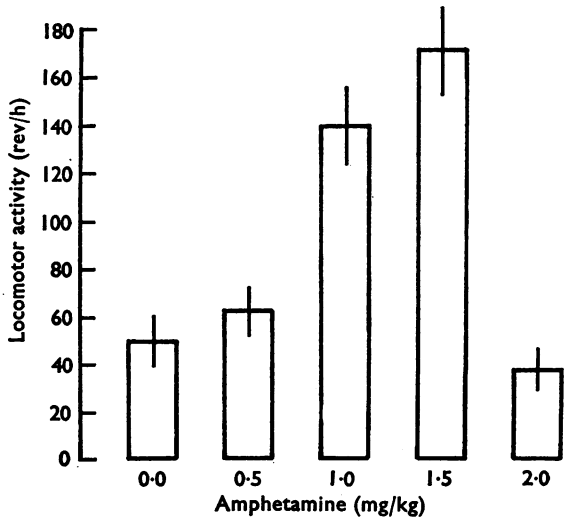

FIG. 1. The effect of amphetamine upon the locomotor activity of C.F.E. rats assessed during the 2 to 6 h period after injection. Each value is the mean of 10 determinations. The vertical bars represent the standard errors on the means.

characterized by periodic sniffing behaviour. At 2·0 mg/kg amphetamine sniffing and constant head movements were exhibited by all animals and the repetition of these movements within a limited area of the activity wheel caused a reduction in forward locomotor activity (Figure 1). A dose of 1·0 mg/kg of amphetamine was selected because it caused a consistent stimulation of locomotor activity but no stereotyped behaviour.

Histological assessment of lesion location

The damage caused by the lesion placed in the substantia nigra was mostly confined to the pars compacta and pars reticulata but slight damage to the dorsal and medially located reticular formation and the lemniscus medialis was frequently observed (Figure 2).

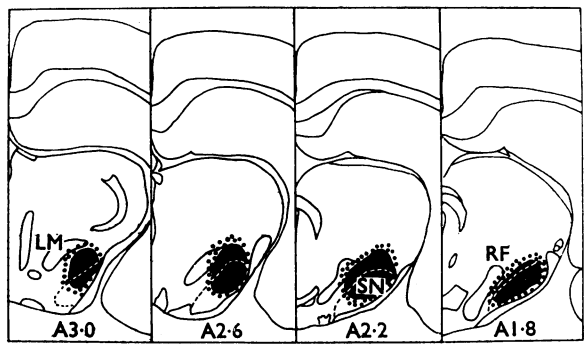

FIG. 2. Diagrammatic representation of the localization and extent of lesion damage in the substantia nigra. The diagrams were constructed from the histological data obtained from the brains of 20 rats. Black shading represents tissue damage common to all rats and stippled shading represents tissue damage observed occasionally in the sections examined. LM—lemniscus medialis. RF—reticular formation. SN—substantia nigra.

It should be noted that the present lesions caused more extensive destruction of the substantia nigra than those previously reported (Costall, Naylor & Olley, 1972b) due to an electrode of larger tip diameter.

Effect of substantia nigra lesions upon locomotor activity

The activity of all animals, operated, sham-operated and control, was found to be high during the first 2 h period, achieving a peak at 1·5 to 2 h, and then falling to a lower, constant level during the following 4 hours. For this reason, activity in all experiments is shown for the two time periods 0 to 2 hours and 2 to 6 hours.

On the 3rd to 4th days following the induction of lesions in the substantia nigra the activity of all rats, C.S.E. or C.F.E., high or low activity responders, was significantly enhanced as compared with that of control animals ($P<0·01$ to $P<0·001$). This increase in activity was maintained for the first 9 post-operative days or, in experiments with low activity rats of C.F.E. strain, for 3 months (Figures 3 and 4).

On the 1st and 2nd postoperative days operated animals from all groups exhibited a spontaneous stereotyped behaviour which was characterized mainly by sniffing

behaviour in low activity animals but also by periodic biting and self-mutilative behaviour in high activity animals.

Effect of substantia nigra lesions upon amphetamine-induced stimulation of locomotor activity

Sham-operated animals

On no occasion were sham-operated animals found to respond to either solvent or amphetamine administration in a manner significantly different from that of the control rats ($P>0{\cdot}05$) although there was a trend for the basal level of activity of the sham-operated animals to be slightly increased. Therefore, for clarity the activities of control animals only are shown in the following figures and the significance of any effect of substantia nigra lesions upon locomotor activity or its modification by amphetamine was assessed by comparison with control rats.

High activity animals

On the 1st day following the induction of lesions in the substantia nigra the response of high activity rats to amphetamine was reduced but this was only

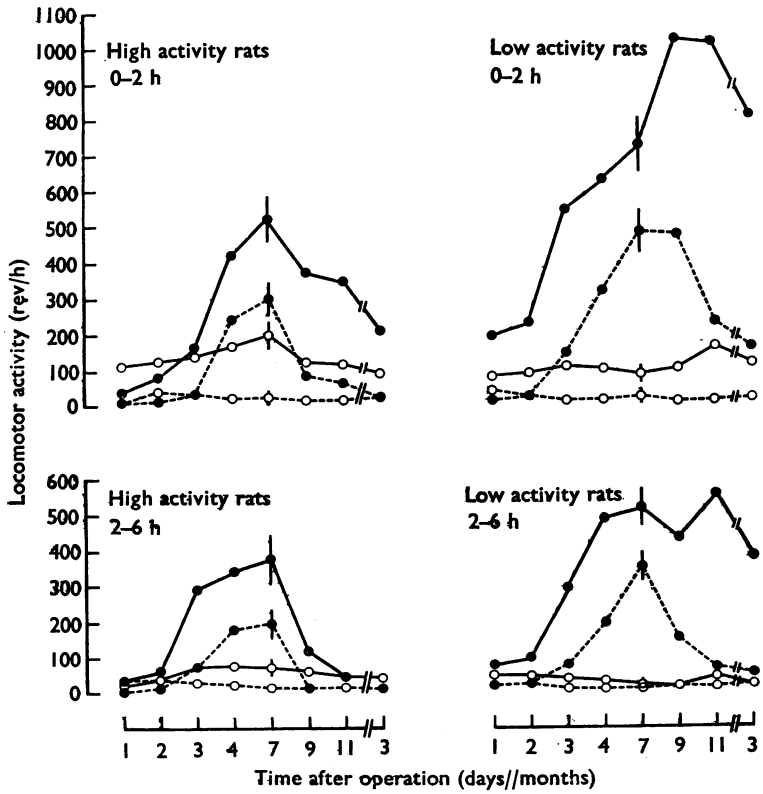

FIG. 3. The effect of bilateral lesions of the substantia nigra upon the stimulation of locomotor activity induced by 1·0 mg/kg amphetamine in C.S.E. rats. ●—Operated animals. ○—Control animals. ---- Solvent. —— Amphetamine. All values given for control animals, and for operated animals on days 1 to 4, are the mean of 15 determinations. Values given for operated animals at other times of assessment are the mean of at least 6 or 9 up to day 11 and 4 or 8 at 3 months for low and high activity animals respectively. The vertical bars represent the standard errors on the means.

significant for the C.S.E. rats during the 0 to 2 h period ($P<0.05$) (Figure 3). After this time the responses of all operated animals to amphetamine increased until the 9th postoperative day, although a comparison of the amphetamine to solvent response ratio for operated and control animals showed no consistent potentiation ($P>0.05$). However, although the stimulant effect of amphetamine in the lesioned animals would appear to decrease on the 11th day and at 3 months, a comparison of the amphetamine: solvent response ratio at these times following the operation shows the stimulant effect of amphetamine to be significantly enhanced in the operated animals of both C.S.E. and C.F.E. strain during the 0 to 2 h period ($P<0.001$) (Figures 3 and 4).

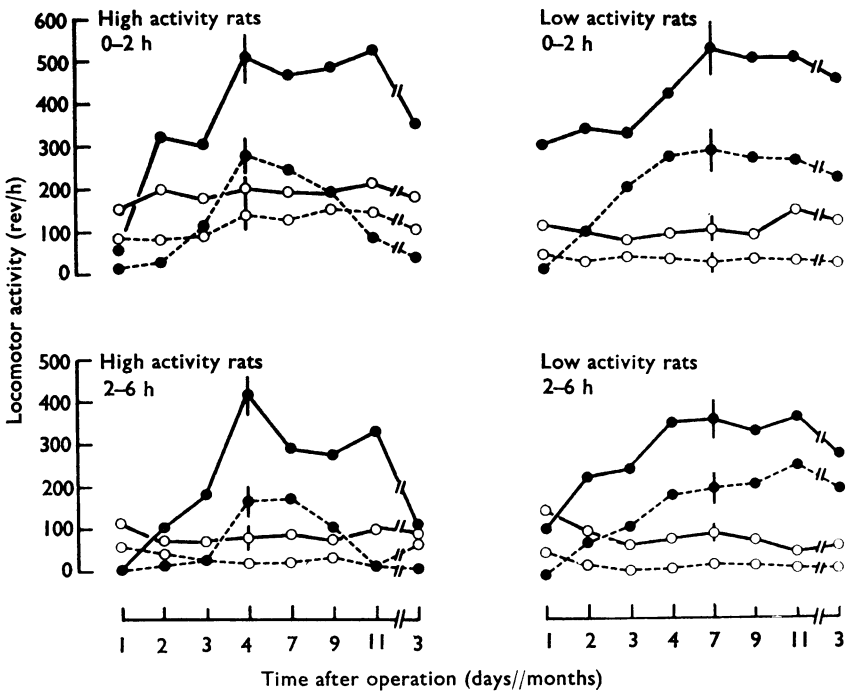

FIG. 4. The effect of bilateral lesions of the substantia nigra upon the stimulation of locomotor activity induced by 1·0 mg/kg amphetamine in C.F.E. rats. ●—Operated animals. ○—Control animals. ---- Solvent. —— Amphetamine. All values given for control animals, and for operated animals on days 1 to 4, are the mean of 15 determinations. Values given for operated animals at other times of assessment are the mean of at least 7 or 11 up to day 11 and 6 or 8 at 3 months for low and high activity animals respectively. The vertical bars represent the standard errors on the means.

Low activity animals

On the first 2 days following ablation of the substantia nigra low activity rats, both C.S.E. and C.F.E. strain, exhibited enhanced responses to the locomotor stimulant effect of amphetamine ($P<0.001$) (Figures 3 and 4). Although on subsequent days of testing there is a trend towards an enhanced response of operated animals to amphetamine, a comparison of the ratio of the amphetamine to solvent responses for the operated and control animals shows this to be insignificant ($P>0.05$) except at 11 days and 3 months for the C.S.E. rats ($P<0.001$) (Figure 3).

Discussion

The substantia nigra is generally considered to exert a facilitative influence upon motor activity via the ascending dopaminergic nigrostriatal pathway (Papeschi, 1972) and ablation of the substantia nigra could, therefore, be expected to cause a reduction in locomotor activity. In the acute stages following lesion of the substantia nigra such a reduction was observed but this could be attributed to the spontaneous stereotypy exhibited by the lesioned animals at this stage (Costall *et al.*, 1972b) and which is shown to reduce locomotor responses. Further, all lesioned animals, whether of high or low activity grouping, C.S.E. or C.F.E. strain, exhibited enhanced locomotor activity levels on the 4th to 7th postoperative days. This would suggest that the substantia nigra, rather than exerting a facilitative effect upon activity, may actually be inhibitory. However, the stimulation following lesion of the substantia nigra was not maintained in all rats and the results of the chronic studies could be forwarded both to support and contradict the work reviewed by Papeschi (1972) for, depending upon the strain of rat and their basal activity level, locomotion was reduced, enhanced or unmodified. Clearly, selection of rats requires careful consideration if results of locomotor activity experiments are to be meaningful, the present results emphasizing the dangers of generalizations in this field.

These comments also apply to the effect of amphetamine upon the activity of the lesioned animals, although in these experiments the generalization can be safely made that the stimulant effect of amphetamine upon locomotor activity was not reduced by ablation of the substantia nigra. This would conflict with the initial findings of Simpson & Iversen (1971) using the electrolytic lesion technique but is in agreement with the studies of Creese & Iversen (1972) in which lesions were induced by 6-hydroxydopamine. Creese & Iversen (1972) showed that, in contrast to a reduction, the locomotor stimulant effect of amphetamine was enhanced following lesion of the substantia nigra. The present studies partially support this view in that all animals, excepting those of low activity and C.F.E. strain, exhibited enhanced responses to amphetamine during the chronic stages following lesion of the substantia nigra. This enhanced stimulation was also apparent during the acute stage following lesions of the substantia nigra but only in the low activity responders of both strains. An explanation of this difference between low and high activity animals may lie in the observation that the spontaneous stereotypy which follows ablation of the substantia nigra, and which is thought to result from a degeneration release of dopamine from the nigrostriatal pathway (Costall *et al.*, 1972b), was marked in the high activity animals, some exhibiting biting and even self-mutilation, whilst the behaviour of the low activity responders was of low intensity characterized mainly by sniffing and repetitive head movements. Whilst the stereotyped behaviour of the high activity animals was of sufficient intensity to prevent any stimulation of locomotor activity the stereotypy induced in the low activity responders was not. Since histologically the lesions induced in the two groups of rats were indistinguishable it is interesting to speculate that some functional difference resides in the substantia nigra and/or the nigrostriatal pathway.

Although the responses of animals to amphetamine have been shown to vary, it is evident from all experiments that the functioning of the substantia nigra is not essential for the locomotor stimulant effect of amphetamine. In previous

studies we have also shown both the neo- and paleostriatum to be non-essential for amphetamine stimulation (Naylor & Olley, 1972). These observations combined suggest that the ability of amphetamine to release dopamine from the nigrostriatal pathway is not related to its locomotor stimulant action.

This work was supported by a grant from the Medical Research Council.

REFERENCES

CARLSSON, A. (1970). Amphetamine and brain catecholamines. In: *International Symposium on Amphetamine and Related Compounds*, Milan, ed. Costa, E. & Garattini, S. pp. 289–300. New York: Raven Press.

COSTA, E., GROPPETTI, A. & NAIMZADA, M. K. (1972). Effects of amphetamine on the turnover rate of brain catecholamines and motor activity. *Br. J. Pharmac.*, **44**, 742–751.

COSTALL, B. & NAYLOR, R. J. (1973). The role of telencephalic dopaminergic systems in the mediation of apomorphine stereotyped behaviour. *Eur. J. Pharmac.* (in press).

COSTALL, B., NAYLOR, R. J. & OLLEY, J. E. (1972a). On the involvement of the caudate-putamen, globus pallidus and substantia nigra with neuroleptic and cholinergic modification of locomotor activity. *Neuropharmacology*, **11**, 317–330.

COSTALL, B., NAYLOR, R. J. & OLLEY, J. E. (1972b). The substantia nigra and stereotyped behaviour. *Eur. J. Pharmac.*, **18**, 95–106.

CREESE, I. & IVERSEN, S. D. (1972). Amphetamine response in rat after dopamine neurone destruction. *Nature, Lond.*, **238**, 247–248.

DE GROOT, J. (1959). The rat brain in stereotaxic co-ordinates. *Verh. K. Ned. Akad. Wet.*, **52**, 1–41.

FAULL, R. L. M. & LAVERTY, R. (1969). Changes in dopamine levels in the corpus striatum following lesions in the substantia nigra. *Expl. Neurol.*, **23**, 332–340.

NAYLOR, R. J. & OLLEY, J. E. (1972). Modification of the behavioural changes induced by amphetamine in the rat by lesions in the caudate nucleus, the caudate-putamen and the globus pallidus. *Neuropharmacology*, **11**, 91–99.

PAPESCHI, R. (1972). Dopamine, extrapyramidal system, and psychomotor function. *Psychiat. Neurol. Neurochir., Amst.*, **75**, 13–48.

SIMPSON, B. A. & IVERSEN, S. D. (1971). Effects of substantia nigra lesions on the locomotor and stereotypy responses to amphetamine. *Nature, Lond.*, **230**, 30–32.

(*Received February* 9, 1973)