Br. J. Pharmac. (1986), **89**, 341–347

Behavioural and adrenocortical responses to nicotine measured in rats with selective lesions of the 5-hydroxytryptaminergic fibres innervating the hippocampus

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1 The effects of acute and subchronic (7) injections of nicotine (0.4 mg kg^{-1}, s.c.) and of selective lesions of the 5-hydroxytryptaminergic (5-HTergic) pathways innervating the hippocampus on the spontaneous behaviour of rats in an elevated X-maze composed of two open and two enclosed runways have been examined.

2 Subchronic, but not acute, nicotine increased total spontaneous activity. Neither acute nor subchronic nicotine altered the ratio of open:closed runway entries.

3 Destruction of the 5-HTergic pathways innervating the hippocampus with 5,7-dihydroxytryptamine caused a reduction in the ratio of open:enclosed runway entries.

4 Acute, but not subchronic, nicotine caused a significant increase in plasma corticosterone. The lesion had no effects on the plasma levels of this hormone.

5 No significant interactions between the lesion and the responses to nicotine were observed.

6 The data failed to provide any evidence that hippocampal 5-HTergic systems may be implicated in the effects of nicotine on the spontaneous behaviour of the rat.

Introduction

Studies reported from a number of laboratories have shown that, whereas acute nicotine administration often results in depression of locomotor activity, its chronic administration generally causes locomotor stimulation (Morrison & Stephenson, 1972; Stolerman *et al.*, 1973; Hendry & Rosecrans, 1982). Benwell & Balfour (1979) have found that rats rapidly develop tolerance to the stimulation of corticosterone secretion evoked by acute injections of nicotine and these authors suggested that its depressant effects on rat behaviour might be directly related to its effects on adrenocortical activity. There is also evidence to suggest that nicotine treatment causes a regionally-selective reduction in the concentration and biosynthesis of 5-hydroxytryptamine (5-HT) in the hippocampus of rat brain (Benwell & Balfour, 1979; 1982a). Other studies have shown that the chronic administration of nicotine attenuates habituation of the adrenocortical response to an aversive stimulus observed when rats are exposed repeatedly to the same aversive environment and that this property of the drug also appears to be associated with regionally-selective changes in hippocampal 5-HT (Benwell & Balfour, 1982b). However, the role of hippocampal 5-HT systems in the mediation of responses to aversive environments remains unclear although Gray (1982) has suggested that decreased 5-HT secretion in the septo-hippocampal system may be implicated in the mechanism by which anti-anxiety drugs reduce behavioural and physiological responses to an aversive stimulus. In this context it is interesting to note that Nelsen (1978) has reported that the administration of nicotine causes an attenuation of the freezing behaviour observed in rats exposed to an aversive stimulus. The purpose of the present study was to examine further the possible role of hippocampal 5-HT systems in the mediation of behavioural responses to nicotine by using the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), to cause selective lesions of the 5-HT pathways which innervate the hippocampus from the median raphe nuclei. The effects of the treatment procedures have been examined by use of an elevated X-maze apparatus which incorporated two open runways as an aversive stimulus. This test

procedure was chosen because it offered a means of detecting both anxiolytic and anxiogenic responses to the treatment protocols used (Pellow *et al.*, 1985) and also allowed the simultaneous measurement of effects on total spontaneous activity.

Methods

Pretreatment procedure

Twenty-four male Sprague-Dawley rats, bred in the Animal Services Unit, Dundee University Medical School from stock purchased from Charles River UK Ltd., and weighing between 250 and 300 g, were given intraperitoneal injections of desmethylimipramine ($10\,mg\,kg^{-1}$). Thirty minutes later they were anaesthetized with equithesin and 5,7-DHT was injected into the pathways innervating the hippocampus from the median raphe nuclei following a protocol very similar to that described by Quik & Azmitia (1983). These pathways represent the principal 5-HTergic innervation into the hippocampus (Bobillier *et al.*, 1976; Azmitia & Segal, 1978). Injections into the fimbria were made at an angle of 13° to the vertical 1.1 to 1.3 mm posterior to the bregma (this co-ordinate being related to the distance between the bregma and lamda co-ordinates), 1.3 mm displaced from the central suture and at a depth of 5.3 mm from the surface of the skull (measured along the injection track). Injections into the two cingulum bundle pathways were also made at 13° with the same AP and lateral co-ordinates but with a vertical co-ordinate of −3.2 mm. Each injection consisted of 5 μg of 5,7-DHT contained in 500 nl of vehicle (0.2 μg ascorbic acid in saline) and was delivered over a period of 2 min. A further 24 rats (sham-operated group) were given microinjections of the vehicle.

Starting 7 days after surgery, 8 of the lesioned rats and 8 of the sham-operated rats were given 6 daily injections of nicotine ($0.4\,mg\,kg^{-1}$, dose expressed as free base) to render them tolerant to the acute effects of the drug and habituate them to the injection protocol (Balfour *et al.*, 1975; Benwell & Balfour, 1979). This dose of nicotine was chosen for the study because previous studies have established that it is behaviourally active (Morrison & Stephenson, 1972; Stolerman *et al.*, 1973) and sufficient to elicit effects on adrenocortical responses to an aversive stimulus (Benwell & Balfour, 1982b). The remaining rats in each group were given subcutaneous injections of saline.

Behavioural measurements

On the day following completion of the pretreatment protocol, both lesioned and sham-operated rats were given subcutaneous injections of nicotine or saline using the schedule summarised in Table 1. Three minutes after the injections each rat was placed at the centre of a symmetrical X-maze raised 1 m from the laboratory floor and consisting of runways which were 45 cm long and 9 cm wide. Two of the runways (the enclosed runways) had sides of 15 cm, the two remaining runways (the open runways) having sides of 3 cm. The maze was constructed of black opaque plastic, the bottom of the runways being covered with removable plywood boards. The numbers of entries made into each of the runways was recorded automatically for 20 min. The rats were then killed immediately by cervical dislocation and brain and blood samples taken for biochemical analysis.

Table 1 Treatment protocol

Intracerebral injections	*Subcutaneous injections* Days 1–6	Day 7
Vehicle	Saline	Saline
Vehicle	Saline	Saline
Vehicle	Nicotine	Nicotine
5,7-DHT	Saline	Saline
5,7-DHT	Saline	Nicotine
5,7-DHT	Nicotine	Nicotine

Each rat was tested in the elevated maze 3 min after the subcutaneous injection on day 7. $n = 8$ per group.
5,7-DHT = 5,7-dihydroxytryptamine.

Biochemical analyses

The plasma corticosterone concentrations were measured by the method of Mattingly (1962). The hypothalamus, hippocampus and cerebral cortex were dissected from each brain by the procedure of Glowinski & Iversen (1966), and the concentrations of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) were measured by the high performance liquid chromatographic procedure with electrochemical detection described by Reinhard *et al.* (1980)

Uptake studies

In a separate experiment, 5 rats were treated with desmethylimipramine and intracerebral injections of 5,7-DHT following the protocol described above. A further 5 rats were given the vehicle. Fourteen days after surgery, the rats were killed and the carrier-mediated uptakes of [^{3}H]-noradrenaline ([^{3}H]-NA) and [^{3}H]-5-HT by synaptosomes were measured in samples of brain homogenate prepared from hypothalamus, hippocampus and cerebral cortex by a method very similar to that described by Balfour (1973). The protein contents of these homogenates were measured

by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Statistical analysis

Both the behavioural and biochemical data were analysed initially by two-way analysis of variance with the intracerebral injections as one factor and the subcutaneous treatment as the second factor. Where appropriate post ad hoc analysis was performed using Newman-Keuls test. The effects of the lesioning procedure on the uptake of [^{3}H]-NA and [^{3}H]-5-HT were analysed by Student's *t* test.

Drugs

Nicotine hydrogen tartrate was purchased from British Drug Houses; 5,7-dihydroxytryptamine creatinine sulphate, 5-hydroxytryptamine creatinine sulphate and 5-hydroxyindole acetic acid from Sigma UK Ltd; [^{3}H]-(+)-noradrenaline (sp. act. 17 Ci mmol^{-1}) and [^{3}H]-5-hydroxytryptamine creatinine sulphate (sp. act. 23.1 Ci mmol^{-1}) from Amersham International.

Results

The injections of 5,7-DHT caused a significant reduction ($P<0.01$) in the carrier-mediated uptake of [^{3}H]-5-HT by homogenates prepared from hippocampus (Table 2). It also appeared to cause a small reduction in [^{3}H]-5-HT uptake by homogenates prepared from cerebral cortex, although this did not reach statistical significance, but had no effect on the uptake by homogenates of hypothalamus. No significant effects on [^{3}H]-NA uptake into any of the brain regions studied were observed. The lesion also caused a marked reduction in the concentrations of 5-HT (F lesion (1,42) = 63.7; $P<0.001$) and 5-HIAA (F lesion (1,42) = 129.8; $P<0.001$) in the hippocampus (Figures 1 and 2). The concentrations of 5-HT (F lesion (1,42) = 4.5; $P<0.05$) and 5-HIAA (F lesion (1,42) = 14.0; $P<0.01$) in cerebral cortex were also reduced by the lesion although the reductions were not as great as those observed in the hippocampus. The lesioned rats also had reduced levels of 5-HIAA (F lesion (1,42) = 11.8; $P<0.01$), but not 5-HT, in the hypothalamus. Nicotine treatment exerted significant effects on the concentration of 5-HIAA in cerebral cortex (F treatments (2,42) = 3.45; $P<0.05$) and the concentration of 5-HT in the hypothalamus (Ftreatments (2,42) = 3.80; $P<0.05$). Nicotine had no effects on the concentrations of 5-HT or 5-HIAA in the hippocampus and there was no statistical evidence to suggest that there were any interactions between the effects of nicotine in the cerebral cortex and hypothalamus and the intracerebral injections of 5,7-DHT.

Analysis of the behavioural data (Figure 3) indicated that treatment with nicotine had a significant effect on the total spontaneous activity of the rats in the maze (F treatment (2,42) = 13.4; $P<0.01$). Further analysis revealed that the rats which had been pretreated with nicotine made significantly more entries (Newman-Keuls; $P<0.01$) than the saline-treated group or the group given acute nicotine. Nicotine exerted effects on entries into both the open (F treatments (2,42) = 7.33; $P<0.05$) and the enclosed runways (F treatments (2,42) = 13.3; $P<0.01$) and, as a result, the drug had no significant effect on the ratio of open:enclosed runway entries (the O/E ratio). In contrast, the lesions appeared to elicit a selective reduction in open runway entries (F lesion (1,42) = 3.90; $P<0.05$) which resulted in a significant reduction (F lesion (1,42) = 8.73; $P<0.05$) in the O/E ratio (Figure 3). None of the interactions between the effects of nicotine and the lesion were significant for any of the behavioural parameters measured.

Analysis of the results of the corticosterone measurements showed that treatment with nicotine exerted a significant effect (F treatments (2,42) = 37.3; $P<0.001$) on the concentration of corticosterone in the plasma (Table 3). The lesion itself had no significant effect on the concentration of the hormone although it did interact with the effect of nicotine

Table 2 The uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) and [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) by synaptosomes prepared from lesioned and sham-operated rats

	[^{3}H]-5-HT uptake (fmol mg^{-1} protein min^{-1})		*[^{3}H]-NA uptake* (fmol mg^{-1} protein min^{-1})	
	Sham-op	*Lesioned*	*Sham-op*	*Lesioned*
Hippocampus	136 ± 10	36 ± 6**	36 ± 4	30 ± 4
Hypothalamus	150 ± 16	168 ± 12	52 ± 8	68 ± 8
Cerebral cortex	128 ± 8	104 ± 10	54 ± 18	62 ± 14

The results are means ± s.e.mean of 5 experiments. Tissue from each animal was assayed in duplicate. The initial concentrations of [^{3}H]-5-HT and [^{3}H]-NA were 10 nM and 15 nM respectively. Significantly different from sham-operated rats: **$P<0.01$.

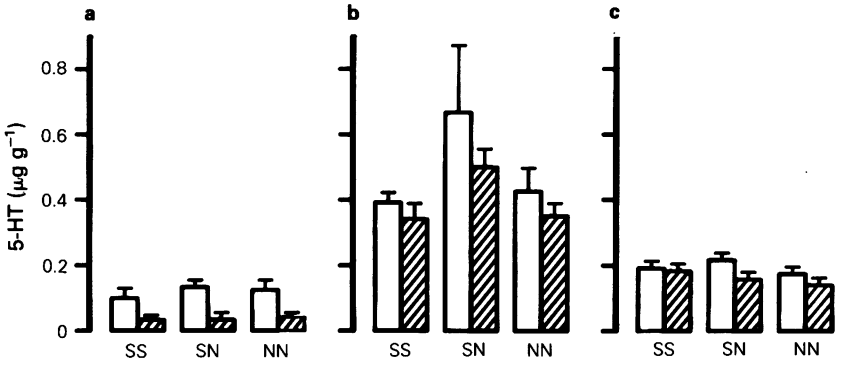

Figure 1 Brain 5-hydroxytryptamine (5-HT) concentrations in sham-operated lesioned rats treated with nicotine or saline: (a) hippocampus, (b) hypothalamus, (c) cerebral cortex. The results are means of 8 observations with s.e.mean shown by vertical lines. Open columns: sham-operated rats; hatched columns: lesioned rats. SS = saline-treated controls; SN = acute nicotine; NN = subchronic nicotine.

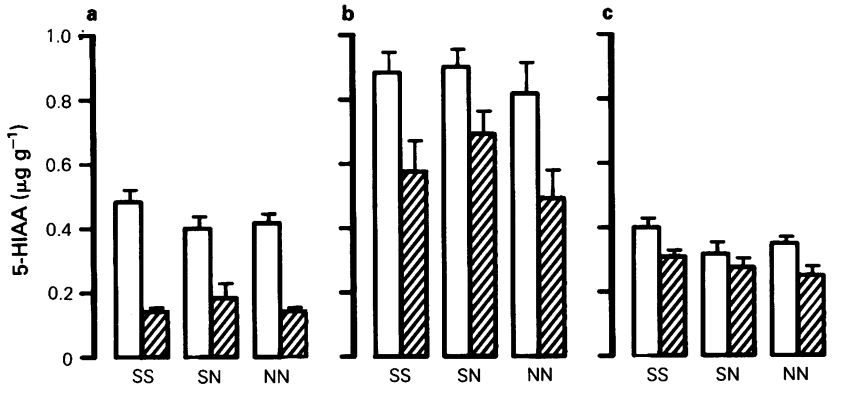

Figure 2 Brain 5-hydroxyindoleacetic acid concentrations in sham-operated and lesioned rats treated with nicotine or saline: (a) hippocampus, (b) hypothalamus, (c) cerebral cortex. The results are means of 8 observations with s.e.mean shown by vertical lines: open columns: sham-operated rats; hatched columns: lesioned rats. SS = saline-treated controls; SN = acute nicotine; NN = subchronic nicotine.

(F interaction (2,42) = 3,57; $P < 0.05$). Subsequent analysis of the data showed that acute injections of nicotine caused a significant increase (Newman-Keuls; $P < 0.01$) in plasma corticosterone when compared with the levels found in rats treated with saline and that this response was unaffected by the lesion. The lesion, however, did significantly attenuate (Newman-Keuls; $P < 0.05$) the development of tolerance to the effects of nicotine on plasma corticosterone observed when the rats were treated subchronically with the drug.

The data were also analysed using a correlation matrix to examine the statistical significance of the linear correlations between the behavioural measures, plasma corticosterone and the concentrations of 5-HT and 5-HIAA in the brain regions studied. The O/E ratio showed a close correlation ($r = 0.88$; $P < 0.01$) with the number of entries made into the open runways and also correlated ($r = 0.65$; $P < 0.01$) with the total spontaneous activity of the rats. The correlation with enclosed runway entries was not significant. The linear correlation coefficients for the relationships between behaviour and brain 5-hydroxyindoles are presented in Table 4. These data reveal no very close relationships between behaviour and the brain 5-hydroxyindole concentrations although both open runway entries and the O/E ratio correlated with the concentrations of 5-HT in all three brain regions examined (cerebral cortex: $P < 0.01$; hippocampus:

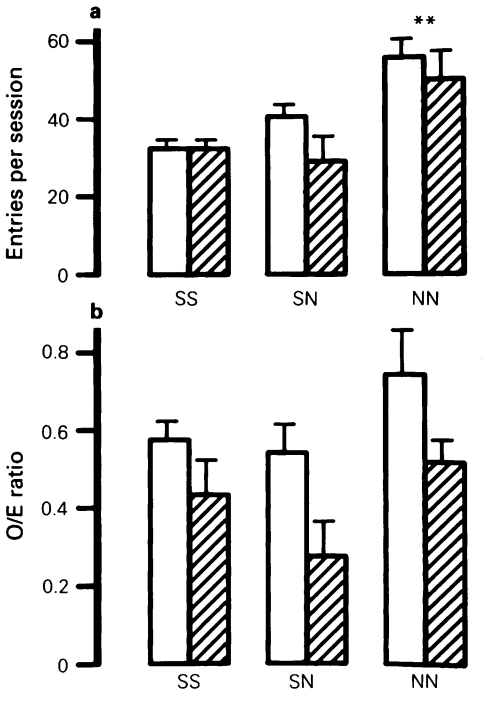

Figure 3 The effects of nicotine and of the lesion on activity in the elevated X-maze: (a) total activity, (b) ratio. The results are means of 8 observations with s.e.mean shown by vertical lines. Open columns: sham-operated rats; hatched columns: lesioned rats. SS = saline-treated controls; SN = acute nicotine; NN = subchronic nicotine.

Table 3 Effects of nicotine on plasma corticosterone in sham-operated and lesioned rats

Treatment	*Plasma corticosterone* (μg 100 ml^{-1}) *Sham-op*	*Lesioned*
Saline	26 ± 1	25 ± 3
Acute nicotine	41 ± 1**	38 ± 1**
Subchronic nicotine	26 ± 1	31 ± 2*

Results are means ± s.e.mean of 8 observations. Significantly different from rats given saline or subchronic nicotine: $^{**}P<0.01$. Significantly different from sham-operated rats: $^{*}P<0.05$.

$P<0.05$; hypothalamus: $P<0.05$). The O/E ratio also correlated ($P<0.05$) with the concentration of 5-HIAA in the cerebral cortex. Both hypothalamic and cerebrocortical 5-HT correlated ($P<0.01$) with total activity. No correlations were observed between plasma corticosterone and either the concentrations of 5-HT and 5-HIAA in the brain or the behavioural parameters measured.

Discussion

The results of the neurochemical studies described in the present investigation clearly indicate that the lesioning procedure used, elicited a selective reduction in the number of 5-HT-secreting nerve terminals in the hippocampus since the injections of the neurotoxin caused a marked reduction in the concentration and carrier-mediated uptake of 5-HT in this region of the

Table 4 Linear correlation coefficients for the relationships between behaviour and the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in discrete regions of rat brain

	Total spontaneous activity	*Enclosed runway entries*	*Open runway entries*	*O/E ratio*
Hippocampal 5-HT	0.26	0.12	0.35*	0.37*
Hippocampal 5-HIAA	0.09	0.03	0.13	0.20
Hypothalamic 5-HT	0.45**	0.28	0.52**	0.40**
Hypothalamic 5-HIAA	0.19	0.20	0.13	0.13
Cerebrocortical 5-HT	0.39**	0.25	0.44**	0.42**
Cerebrocortical 5-HIAA	0.21	0.09	0.28	0.37*

$^{*}P<0.05$; $^{**}P<0.01$.

brain, while having comparatively little or no effect on these parameters in the other two brain regions studied. The specificity of the procedure was also demonstrated by the fact that no significant changes in the uptake of [^{3}H]-NA were observed. Interestingly, however, the effects of the lesions on the concentrations of 5-HIAA were not restricted to the hippocampus although they did appear to be greatest in this region of the brain. Speisky & Kalant (1985) have reported that combined lesions of the 5-HT pathways of the cingulum bundle and fornix/fimbria cause some reduction of the 5-HT and 5-HIAA concentrations of the cerebral cortex, results that are clearly in agreement with the data reported in the present investigation. However, since consistent reductions in 5-HIAA were observed in both hypothalamus and cerebral cortex of lesioned rats, it is perhaps more likely that, in these rats at least, the loss of 5-HT secreting terminals in the hippocampus may have been associated with reduced 5-HT secretion in these other two brain regions.

The data reported here for the effects of nicotine on the behaviour of the rats in the maze are in very good agreement with the results of previous studies with unoperated rats in this maze (Balfour *et al.*, 1986). Specifically both this and the earlier study have shown the subchronic, but not acute, nicotine causes a stimulation of spontaneous activity while having no effect on the O/E ratio. This study, however, has also shown that lesions of the hippocampal 5-HT system cause a decrease in the O/E ratio, a response which the results of previous studies (Handley & Mithani, 1983; Pellow *et al.*, 1985) would suggest is indicative of an anxiogenic effect. The results described here are perhaps surprising since Gray (1982) has proposed that anxiolytic drugs may attenuate responses to aversive stimuli by reducing 5-HT secretion in the septo-hippocampal system. Plaznik *et al.* (1983) have also reported that microinjections of 5-HT into the rat hippocampus cause changes in behaviour which suggest that the injections mimic or enhance the effects of anxiogenic stimuli. However, other groups have reported that benzodiazepine anti-anxiety drugs appear to increase 5-HT secretion in the brain (Agarwal *et al.*, 1979; Collinge & Pycock, 1982). The present data have also indicated that lesioning the hippocampal 5-HT system results in changes in 5-hydroxyindole levels in other regions of the brain and that the correlations between open runway entries and brain 5-HT were not restricted to the hippocampus. Therefore the relationship between the lesions in the hippocampus and the changes in behaviour in the maze may be complex in nature.

The results have shown that there were no interactions between the lesion and the effects of nicotine on rat behaviour in the maze. They have also shown that, although open entries and the O/E ratio correlated significantly with hippocampal 5-HT, better correlations between these behavioural parameters and the concentrations of 5-HT in hypothalamus and cerebral cortex were observed. Thus the study would suggest that hippocampal 5-HT systems are not implicated specifically in the mechanism by which subchronic nicotine stimulates spontaneous activity in the rat. The lesion did, however, appear to inhibit the development of tolerance to the effects of nicotine on plasma corticosterone. The data currently available cannot provide an explanation for this observation although it is interesting to note that Azmitia *et al.* (1984) have recently reported that the 5-HTergic fibres which innervate the hippocampus appear to synapse with the neurones which bind corticosterone. These neurones are reported to be involved in the mechanism by which rats terminate the adrenocortical response to a stressful stimulus (Sapolsky *et al.*, 1984). The same neurones may also mediate the development of tolerance to the effects of nicotine on adrenocortical activity and, if this is the case, the data reported here suggest that hippocampal 5-HT systems may also be implicated in this process. Clearly, however, further studies are necessary to establish this unequivocally.

Previous studies (Balfour *et al.*, 1986) have shown that the increase in O/E ratio observed in rats given diazepam appears to be associated with a reduction in the plasma corticosterone concentration. These earlier experiments also showed that the increased plasma corticosterone levels observed in rats given acute nicotine were not associated with a decrease in the ratio. This latter observation has been confirmed in the present study which, in addition, has also shown that the apparent anxiogenic response to the lesioning procedure was also not associated with increased plasma corticosterone. Thus the data are clearly in agreement with previous results which indicated that, in certain test situations at least, plasma corticosterone levels may not necessarily be a good measure of the anxiolytic or anxiogenic properties of a drug.

This study was performed with financial support from the M.R.C. and the Scottish Home and Health Department.

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(Received March 3, 1986.
Revised May 3, 1986.
Accepted May 22, 1986.)