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Studies on the possible role of brain 5-HT systems and adrenocortical activity in behavioural responses to nicotine and diazepam in an elevated X-maze

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Abstract. Subchronic (6 days) but not acute injections of nicotine (0.4 mg/kg SC) increased spontaneous activity ($P<0.01$) in an elevated X-maze composed of two open and two enclosed runways. Neither acute nor subchronic nicotine altered significantly the ratio of open: enclosed runway entries (O/E ratio). Diazepam (5 mg/kg PO) had no significant effects on spontaneous activity but increased the O/E ratio ($P<0.05$). Acute nicotine increased ($P<0.01$) whereas subchronic nicotine caused a small decrease ($P<0.05$) in the plasma corticosterone concentration. Both acute and subchronic diazepam decreased the levels of the hormone ($P<0.01$ and $P<0.05$, respectively) although the reduction elicited by chronic diazepam was less than that caused by acute diazepam ($P<0.05$). In the experiments with diazepam the plasma corticosterone concentration correlated negatively with the O/E ratio ($r=-0.58$; $P<0.05$), whereas in the experiments with nicotine plasma corticosterone correlated negatively ($r=-0.46$; $P<0.05$) with enclosed runway entries. Nicotine injections were associated with a regionally-selective reduction in the 5-hydroxyindole acetic acid (5-HIAA) concentration in the hippocampus ($P<0.05$) and a reduction in hippocampal 5-hydroxytryptamine (5-HT) which approached statistical significance. Chronic, but not acute, diazepam increased ($P<0.01$) hypothalamic 5-HT. The changes in 5-HT and 5-HIAA did not appear to be directly related to the behavioural or adrenocortical responses to either of the drugs.

Key words: Nicotine – Diazepam – Spontaneous activity – Elevated X-maze – Brain 5-hydroxyindoles – Plasma corticosterone – Rats

In their recent review, Hendry and Rosecrans (1984) summarised evidence which indicated clearly that the behavioural effects of nicotine were influenced to a considerable extent by the environmental stimuli associated with the test procedure used and the duration of drug treatment prior to the test day. In particular, it has been shown that, if nicotine is administered subcutaneously at doses in excess of 0.4 mg/kg, acute injections of the drug cause a reduction in spontaneous activity whereas chronic injections increase spontaneous activity (Keenan and Johnson 1972; Morrison and Stephenson 1972; Stolerman et al. 1973). The effects of nicotine on unconditioned avoidance of an aversive stimulus appear to be equivocal, since the drug has been shown to increase low levels of avoidance but to decrease high avoidance levels of the open runway of a Montgomery Y-maze (Morrison and Stephenson 1970). Nicotine also does not exert consistent effects on conditioned avoidance behaviour, although it has a tendency to increase the rate of aquisition of avoidance responses (Hendry and Rosecrans 1984). In addition, Morrison (1974) has reported that nicotine withdrawal from rats trained on a Sidman avoidance schedule under its influence causes disruption of avoidance behaviour and that the degree of disruption appears to be related to the degree of stress associated with the procedure. Balfour (1984) has subsequently summarised evidence which suggests that this effect of nicotine withdrawal on avoidance behaviour may be related to the ability of the drug to attenuate the disruptive effects of aversive stimuli on behavioural activity.

In other studies Benwell and Balfour (1979) have shown that rats rapidly develop tolerance to the stimulation of corticosterone secretion observed when unstressed rats are treated acutely with nicotine. In contrast, nicotine attenuates the reduction in plasma corticosterone which occurs when rats habituate to an aversive environment (Benwell and Balfour 1982a). Nicotine injections to unstressed rats have also been shown to cause a regionally selective reduction in the concentration and biosynthesis of 5-hydroxytryptamine (5-HT) in the hippocampus of rat brain (Benwell and Balfour 1979, 1982b). The effects of nicotine on the processes associated with adaptation to an aversive stimulus also appear to be associated with regionally-selective changes in hippocampal 5-HT (Benwell and Balfour 1982a). However, although the possible relationships between the changes in plasma corticosterone and brain 5-HT, evoked by nicotine, and behavioural responses to the drug have been the subject of some speculation (Benwell and Balfour 1979, 1982a; Balfour 1984), there appear to be few reports of studies which have been designed specifically to investigate the relationships between behaviour, brain 5-HT and adrenocortical activity in rats treated with nicotine. The purpose of the present study was to examine these relationships using a maze apparatus designed to measure both changes in spontaneous activity and anxiolytic or anxiogenic responses to the drug, and to compare the results obtained with those seen in response to the established anxiolytic drug, diazepam.

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Methods

Animals. The animals used for the study were male Sprague-Dawley rats, bred in the Animal Services Unit, Dundee University Medical School from stock purchased from Charles River UK Ltd. and weighing approximately 250 g at the beginning of the experiment. During the pretreatment schedule they were housed in pairs in a room lit between 0800 and 2000 hours each day and allowed free access to food and water.

Treatment protocol. In the studies with nicotine, three groups of rats ($n=8$ per group) were given daily subcutaneous injections of saline (two groups) or nicotine (0.4 mg/kg; one group) for 6 days. They were returned immediately to their home cages following each injection. The nicotine was administered in the form of its hydrogen tartrate, the dose being expressed in terms of the free base. On day 7, the rats in the group pretreated with nicotine and one of the groups pretreated with saline were given nicotine 3 min prior to being placed at the centre of an elevated X-maze. The animals in the remaining group were given saline prior to being placed in the maze. The maze, which was placed on a frame which raised it 1 m from the laboratory floor, was composed of four runways which were 45 cm long and 9 cm wide. Two opposing runways (the enclosed runways) had sides of 15 cm; the other two runways (the open runways) had sides of 3 cm. In this type of maze anxiolytic drugs are reported to increase the ratio of open:enclosed runway entries (O/E ratio) whereas anxiogenic drugs decrease the ratio (Pellow et al. 1985). The numbers of entries made by the rats into each of the arms of the maze were recorded automatically as 4-min subtrials for 20 min. The rats were then killed by cervical dislocation and brain and blood samples taken for biochemical analysis. All the experiments were performed between 1230 and 1400 hours.

Studies with diazepam. The effects of diazepam on behaviour in the maze and on plasma corticosterone levels were examined using three additional groups of rats ($n=6$ per group). The animals in one group were pretreated daily with diazepam (5 mg/kg), administered by orogastric intubation, for 6 days, the remaining two groups being given the vehicle (40% (v:v) propylene glycol in water). On day 7 the group pretreated with diazepam and the rats in one of the other groups were given diazepam and 30 min later they were placed at the centre of the elevated X-maze and the entries into each arm recorded for 20 min. The animals in the remaining group were given the vehicle prior to being tested in the maze. All the rats were killed immediately at the end of the trial and brain and blood samples taken from each for biochemical analysis.

Biochemical analysis. Plasma corticosterone was measured using the method of Mattingly (1962) but adapted for small plasma volumes. The concentrations of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) in hippocampus, hypothalamus and cerebral cortex were measured using the high pressure liquid chromatography procedure described in Reinhard et al. (1979).

Statistical analysis. Both behavioural (data for total sessions) and biochemical data were analysed initially by one-way analysis of variance followed, when appropriate, by Newman-Keuls test to determine the significance of the differences between the individual groups in the study. A MANOVA for repeated measures was used to examine the effects of the subtrials on behavioural responses to the drugs. The method of least squares was used to determine the linear correlations between the biochemical parameters and the measures of rat behaviour.

Results

Rats treated with saline or the diazepam vehicle made significantly more entries ($P<0.01$) into the enclosed runways than the open runways and, as a result, the O/E ratio was consistently less than unity (Figs. 1 and 2). Diazepam tended to increase the total activity of the rats, although this effect was not statistically significant for rats treated either acutely or subchronically with the drug. Diazepam did, however, exert a significant effect [$F(2,15)=4.1$; $P<0.05$] on the O/E ratio (Fig. 1). Analysis using Newman-Keuls test showed that both acute and subchronic diazepam increased ($P<0.05$) the ratio. Analysis of the subtrials (Fig. 3) revealed that the spontaneous activity of the rats changed significantly during the course of the trial [F subtrials $(4,60)=13.1$; $P<0.001$]. Diazepam did not interact significantly with this effect. The changes in the O/E ratio which occurred as the trial progressed were not significant. However, the interaction between the effects of diazepam on the O/E ratio and subtrials was highly significant [F treatment by subtrials $(8,60)=3.2$; $P<0.01$]. An examination of the data (Fig. 3) would indicate that although both acute and subchronic diazepam appeared to increase the ratio in the first part of the trial, the greatest effect of subchronic diazepam was observed during the last subtrial whereas this was not the case when the drug was given acutely.

Diazepam treatment exerted a significant effect on the concentration of corticosterone in the plasma [$F(2,15)=21.8$; $P<0.01$]. Acute diazepam decreased the levels from 26.6 ± 1.1 to 15.5 ± 0.6 µg/100 ml (Newman-Keuls $P<0.01$). Subchronic diazepam also decreased plasma corticosterone (Newman-Keuls $P<0.05$) to 20.3 ± 2.1 µg/100 ml. This concentration, however, was significantly higher (Newman-Keuls $P<0.05$) than that measured in the rats given the drug acutely. The correlation coefficient between plasma corticosterone and the O/E ratio ($r=-0.58$; $n=18$) was statistically significant ($P<0.05$), whereas the correlation coefficients between plasma corticosterone and the other measures of activity in the maze (total spontaneous activity, open and enclosed runway entries) did not reach statistical significance.

Analysis of the effects of drug administration on the concentrations of 5-HT and 5-HIAA in the brain showed that diazepam exerted effects on the concentration of 5-HT in the hypothalamus [$F(2,15)=12.2$; $P<0.01$]. Subsequent analysis with Newman-Keuls test showed that subchronic diazepam increased ($P<0.01$) the concentration of hypothalamic 5-HT from $0.315+0.055$ to 0.835 ± 0.130 µg/g. Acute diazepam had no effect. Diazepam also had no significant effects on the concentrations of 5-HT in either of the other two brain regions studied or on the concentrations of 5-HIAA in any of the regions examined. No significant linear correlations between the brain 5-hydroxyindole levels and the plasma corticosterone concentration or any of the measures of behaviour were observed.

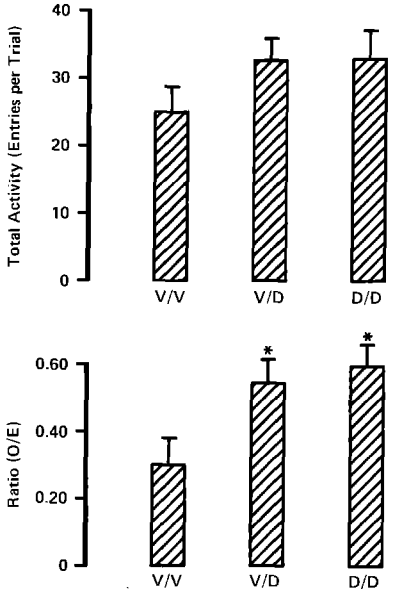

Fig. 1. Effects of diazepam on spontaneous activity in the elevated X-maze. The measurements were made with rats treated orogastrically with vehicle (*V/V*), acute (*V/D*) or subchronic (*D/D*) diazepam (5 mg/kg). The results are means ± SEM of six independent observations. * Significantly different from vehicle-treated rats ($P<0.05$)

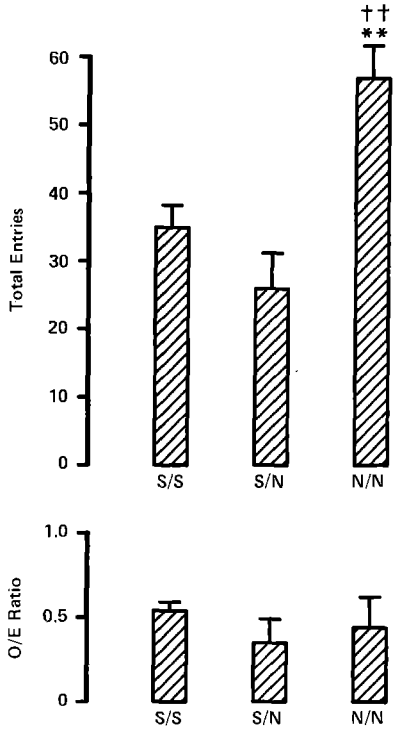

Fig. 2. Effects of nicotine on spontaneous activity in the elevated X-maze. The measurements were made with rats treated subcutaneously with saline (*S/S*), acute (*S/N*) or subchronic (*N/N*) nicotine (0.4 mg/kg). The results are means ± SEM of eight independent observations. ** Significantly different from saline-treated rats ($P<0.01$); ++ significantly different from rats treated with acute nicotine ($P<0.01$)

Total spontaneous activity was affected significantly by treatment with nicotine [$F(2,21)=17.0$; $P<0.001$]. The acute administration of the drug had no effect on spotaneous activity, whereas the rats which had been pretreated

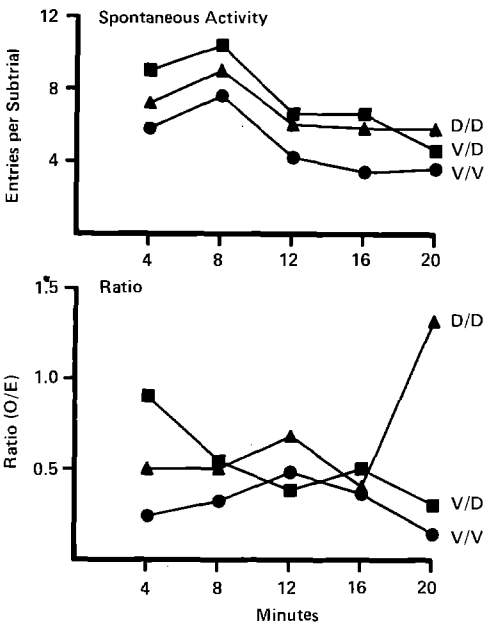

Fig. 3. Effects of diazepam on spontaneous activity: subtrial data. The measurements were made in rats treated orgastrically with vehicle (*V/V*), acute (*V/D*) or subchronic (*D/D*) diazepam (5 mg/kg). The results are means of six independent observations

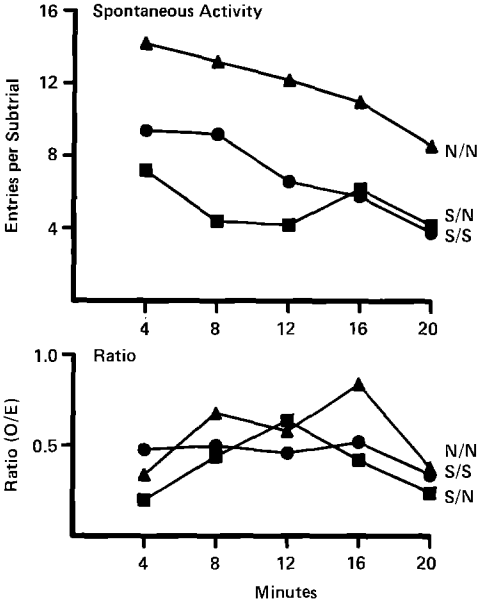

Fig. 4. Effects of nicotine on spontaneous activity: subtrial data. The measurements were made with rats treated subcutaneously with saline (*S/S*), acute (*S/N*) or subchronic (*N/N*) nicotine (0.4 mg/kg). The results are means of eight independent observations

with nicotine exhibited significantly increased (Newman-Keuls $P<0.01$) spontaneous activity (Fig. 2). Nicotine had no significant effects on the O/E ratio. Analysis of the data for the individual subtrials (Fig. 4) revealed a significant effect of subtrials on the spontaneous activity of the rats [F subtrials $(4,84)=10.5$; $P<0.001$]. Nicotine did not interact significantly with this effect. The effect of subtrials on the O/E ratio was not significant neither was the interaction between subtrials and nicotine treatment.

The administration of nicotine resulted in significant

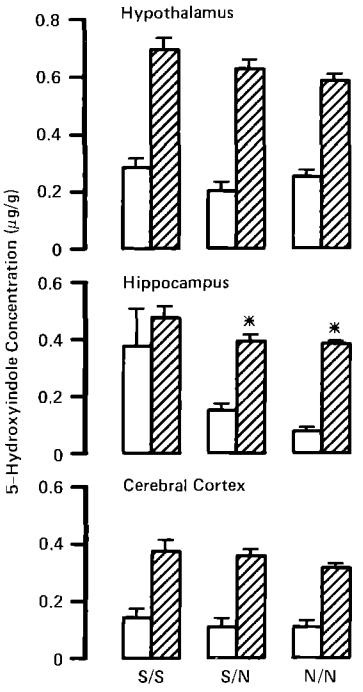

Fig. 5. Effects of nicotine on the concentrations of 5-HT and 5-HIAA in specific regions of rat brain. The concentrations of 5-HT(□) and 5-HIAA (▨) were measured in brain samples taken from rats treated with saline (*S/S*), acute (*S/N*) or chronic (*N/N*) nicotine (0.4 mg/kg) prior to being tested in the elevated X-maze. The results are means ± SEM of eight observations. * Significantly different from value measured in rats given saline

Table 1. Linear correlations between the behavioural and biochemical measurements

	Open entries	Enclosed entries	Total activity	O/E ratio
Plasma corticosterone	−0.233	−0.459*	−0.394	0.128
Hypothalamic 5-HT	−0.139	0.037	−0.049	−0.175
Hypothalamic 5-HIAA	−0.126	−0.476*	−0.349	0.172
Hippocampal 5-HT	−0.161	−0.197	−0.200	−0.072
Hippocampal 5-HIAA	−0.267	−0.213	−0.265	−0.239
Cerebrocortical 5-HT	0.002	−0.169	−0.100	0.097
Cerebrocortical 5-HIAA	−0.364	0.170	−0.289	−0.268

* $P < 0.05$

changes [$F(2,21) = 34.0$; $P < 0.001$] in the plasma corticosterone concentration. Acute nicotine caused a significant increase (Newman-Keuls $P < 0.01$) in plasma corticosterone from 22.3 ± 1.3 to 35.8 ± 1.7 μg/100 ml, whereas subchronic nicotine reduced the concentration to 19.11 ± 1.5 μg/100 ml, a level which was significantly lower (Newman-Keuls) than those found in rats given acute nicotine ($P < 0.01$) or saline ($P < 0.05$). Nicotine treatment also caused a significant change [$F(2,21) = P < 0.01$] in the concentration of 5-HIAA in the hippocampus (Fig. 5). Subsequent analysis showed that both acute and subchronic nicotine caused a reduction (Newman-Keuls $P < 0.05$) in the concentration of this 5-hydroxyindole. Both acute and subchronic nicotine also appeared to reduce the concentration of 5-HT in this region of the brain. This effect, however, only approached statistical significance. Nicotine had no significant effects on the concentrations of 5-HT or 5-HIAA in the other two brain regions examined.

Analysis of the linear correlations between the biochemical and behavioural measurements made in the rats treated with nicotine or saline showed that there were significant ($P < 0.05$) negative correlations between the number of entries made into the enclosed runways and both the plasma corticosterone concentration and the concentration of 5-HIAA in the hypothalamus (Table 1). No statistically significant linear correlations between open runway entries or the O/E ratio and the biochemical measures were observed. However, there was a significant negative correlation ($r = -0.445$; $P < 0.05$) between plasma corticosterone and the concentration of 5-HT in the hypothalamus.

Discussion

The results of the present investigation have shown that; whereas the acute injections of nicotine had no significant effects on the spontaneous activity of the rats in the X-maze, its subchronic administration caused increased locomotor activity. These data are in good accord with results reported from other laboratories for the effects of acute and subchronic nicotine on spontaneous activity in open fields, Y-mazes and tunnel mazes (Morrison and Stephenson 1972; Stolerman et al. 1973; Bättig et al. 1975). The effects of nicotine on plasma corticosterone, reported here, are also in agreement with the results of previous studies with unstressed rats (Benwell and Balfour 1979) which showed that rats treated chronically with nicotine rapidly develop tolerance to the stimulation of adrenocortical activity observed when the drug is given acutely. These data would, therefore tend to support the hypothesis, first proposed by Benwell and Balfour (1979), that the stimulant effects of nicotine on spontaneous behaviour do not become apparent until the rats have become tolerant to the acute effects of the drug on corticosterone secretion.

Reports from other laboratories (Handley and Mithani 1983; Pellow et al. 1985) have shown that rats treated with anxiolytic drugs exhibit an increased O/E ratio when tested in an elevated X-maze very similar in design to that used in the present study, whereas anxiogenic drugs tend to decrease the ratio. Indeed, Pellow and her colleagues (1985) have suggested that an increased O/E ratio may be a characteristic response to anxiolytic drugs in this apparatus. Results reported here have confirmed the effects of diazepam on the O/E ratio, although they suggest that, for acute diazepam, the effect does not persist for the full 20-min trial used in this study. The results also suggest that, in this apparatus at least, nicotine does not exert anxiolytic or anxiogenic effects. This could reflect the dose of nicotine chosen for the study although it was clearly sufficient to elicit a marked increased in spontaneous activity of approximately 55% when given subchronically and, in previous studies (Benwell and Balfour 1982a), to interact with the process by which rats adapt to aversive stimuli. Morrison and Stephenson (1970) found that when nicotine, administered at the same dose as that used in the present investigation, elicited a similar increase in spontaneous activity, it also appeared to show anxiolytic effects. Clearly, the present study does not support this conclusion.

The benzodiazepine anti-anxiety drugs have been shown to reduce the raised plasma corticosterone levels measured in rats exposed to stressful environments (Le Fur et al.

1979; Keim and Sigg 1977). These studies have shown that diazepam also exerts this effect in rats tested in the elevated X-maze, although results suggest that rats treated subchronically with the drug may have developed some tolerance to this response. The effects of subchronic diazepam and nicotine on the plasma corticosterone levels were somewhat similar, although the reduction elicited by nicotine was very small. However, diazepam administration was associated with an increased O/E ratio which correlated significantly with reduced plasma corticosterone, whereas subchronic nicotine increased total activity but had no effect on the O/E ratio. Acute injections of nicotine increased plasma corticosterone but also had no effect on the O/E ratio. Indeed, in contrast to the results obtained with rats treated with diazepam or its vehicle, the effects of nicotine on plasma corticosterone correlated best with the number of entries made into the enclosed runways rather than the O/E ratio. Thus it would appear that the effects of drugs on plasma corticosterone do not necessarily reflect an anxiolytic or anxiogenic property of the compound.

The study has shown that subchronic, but not acute, treatment with diazepam caused a significant increase in the concentration of 5-HT in the hypothalamus. The possible pharmacological significance of this effect remains unclear, although the data presented here would suggest that it is probably not related directly to the anxiolytic properties of the drug since both acute and subchronic diazepam increased the O/E ratio and decreased the plasma corticosterone concentration. It is possible that the change in hypothalamic 5-HT could be associated with the apparent development of tolerance to the effects of diazepam on adrenocortical activity, although clearly further studies are required to establish this with any certainty.

In contrast to the results with diazepam, treatment with nicotine had no effects on hypothalamic 5-HT but caused a significant reduction in the concentration of 5-HIAA in the hippocampus. Since the reduction in hippocampal 5-HT observed in the nicotine-treated rats also approached significance, it seems reasonable to suggest than these changes in the hippocampus may reflect a regionally-selective reduction in 5-HT turnover in this region of the brain. Interestingly, previous experiments with unstressed rats (Benwell and Balfour 1979, 1982b) have also shown that nicotine injections cause a regionally-selective reduction in the concentration and biosynthesis of 5-HT in the hippocampus. However, the present study has not provided any clear indication of the possible role of hippocampal 5-HT systems in the effects of nicotine on plasma corticosterone or spontaneous behaviour since the results, both with nicotine and diazepam, revealed no obvious relationships between the concentrations of 5-HT or 5-HIAA in this region of the brain and the adrenocortical or behavioural activity of the animals. Recently, FitzGerald and his colleagues (1985) have reported that depletion of whole brain 5-HT with *para*-chlorophenylalamine (*p*CPA) caused a significant reduction in nicotine-induced hyperactivity, measured in a tunnel maze, whereas it had no significant effects on the spontaneous activity of saline-treated controls. These data are clearly inconsistent with the hypothesis that nicotine stimulates spontaneous activity by decreasing 5-HT secretion in the brain. However, these authors also reported that pretreatment with 5-hydroxytryptophan, did not reverse the effects of pCPA in the nicotine-treated rats and, indeed, caused reduced activity in both control and drug-treated animals. As a result, they concluded that brain, although not specifically hippocampal 5-HT systems, appear to participate in the stimulant response to nicotine but that their location and function remain unclear. The results reported in this paper are entirely consistent with this conclusion.

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