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A Possible Role for the Pituitary-Adrenal System in the Effects of Nicotine on Avoidance Behaviour[1]

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BALFOUR, D. J. K. AND C. F. MORRISON. *A possible role for the pituitary-adrenal system in the effects of nicotine on avoidance behaviour.* PHARMAC. BIOCHEM. BEHAV. 3(3) 349–354, 1975. — Rats were trained on a Sidman lever-pressing avoidance task. Half the animals received 0.4 mg nicotine/kg before each avoidance session. Rats which were successful performers had heavier adrenal glands. Nicotine facilitated avoidance and increased adrenal weight. Successful avoiders also tended to have higher hippocampal 5-HT levels. Hypothalamic 5-HT was elevated in animals which had just received many shocks during the final avoidance session but not in rats which had received a comparable number of unavoidable shocks. No effects of nicotine on noradrenaline or corticosterone were detected nor were these measures related to avoidance performance.

Chronic nicotine injections   Noradrenaline   5-Hydroxytryptamine   Adrenal weight   Corticosterone
Avoidance behavior

CONSIDERABLE evidence is now available for a relationship between avoidance behavior and pituitary-adrenal function [4, 14, 15, 16]. Nicotine facilitates the acquisition and performance of lever-pressing avoidance in the rat [3, 5, 10] and also increases plasma corticosterone levels in the same species [2,7]. It therefore seemed possible that the effects of nicotine on avoidance might, at least in part, result from activation of the pituitary-adrenal system. In the present experiments an attempt has been made to relate avoidance performance to changes in the levels of plasma corticosterone and of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) in the hippocampus and hypothalamus, brain regions which have been implicated in the control of the pituitary-adrenal response [8].

METHOD

Animals

Thirty-six male rats, 14 Sprague-Dawley and 22 cross-bred (F1 of a Sprague-Dawley, Lister black-hooded cross) were used in the avoidance experiments. Their ages at the end of the experiment ranged from 147–220 days (mean 175) and they weighed from 381–659 g (mean 507 g). In addition 24 male cross-bred rats were used in the experiment involving unavoidable shocks and a further 24 male rats (12 Sprague-Dawley, 12 cross-bred) were used in the experiment in which rats received nicotine but no avoidance training.

Avoidance Training

The rats were trained in Grason-Stadler 2-lever rat chambers on a Sidman [13] avoidance schedule. During training electric shocks of 0.5 sec duration were delivered through the grid floor every 30 sec. Each lever-pressing response postponed the next shock for 30 sec. Initially the rats were trained to press on a continuous reinforcement (crf) schedule for water. After a few sessions, during which crf and avoidance were programmed simultaneously, the water reinforcements were discontinued. Shock levels were gradually increased from 0.1–0.4 mA. This training took 13–31 days for different groups of rats and was followed by 20–35 days on the experimental schedule. In this the shocks were delivered every 30 sec and each time the rat pressed the lever the next shock was postponed for 30 sec.

Procedure

The rats were trained and tested in groups of 6, each

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consisting of 3 littermate pairs. Each rat was run singly for 60 min at the same time each day for 5 days a week. Animals of a pair were run in the same chamber during consecutive 60 min sessions.

One rat of each pair received 0.4 mg nicotine/kg subcutaneously immediately before each avoidance session (nicotine trained). On 4 occasions at 1 week intervals a saline injection was substituted for the nicotine. The other rat received saline before each session (saline trained). The nicotine injections consisted of nicotine hydrogen tartrate dissolved in 0.9% saline to give an injection volume of 0.1 ml/100 g body weight. The dose is expressed as base. One saline trained Sprague-Dawley rat died during training and results are presented for 35 rats only.

Nine of the nicotine trained rats received nicotine and 9 received saline immediately before the session on the final day of the experiment.

Unavoidable Shocks

The rats were assigned to groups of 3 and placed singly in Grason-Stadler rat chambers for 60 min. One rat from each group received 48 shocks (0.5 sec, 0.4 mA) at regular intervals during the 60 min (high shock), the second rat received 10 shocks (low shock) and the third rat was unshocked.

Chronic Nicotine

Six Sprague-Dawley rats and 6 cross-bred rats received a single injection of nicotine (0.4 mg/kg) for 5 consecutive days for 5 weeks (25 injections in all), but no avoidance training. Littermate controls, 6 Sprague-Dawley and 6 cross-bred, received saline in place of nicotine, 24 hr after the last injection, the adrenal glands were removed, cleaned and weighed.

Biochemical Procedure

Immediately after the final avoidance or unavoidable shock session the rats were killed by decapitation. Plasma corticosterone was estimated using the method of Mattingly [9] as described by Balfour *et al.* [2]. Hippocampal and hypothalamic 5-HT and NA were estimated using the method of Ansell and Beeson [1] as modified by Balfour *et al.* [2]. The adrenal glands were removed, cleaned of extraneous fat and weighed.

Statistics

The Wilcoxon matched-pairs, signed-ranks test was used to assess differences between treatments, using results pooled from both strains of rat. The rat with no partner was omitted. Strain differences within treatment groups were tested by the Mann-Whitney U-test. A linear correlation matrix was computed for the avoidance experiment using all the biochemical data, various measurements of avoidance performance, adrenal weight, body weight and age. The results in the text are expressed as means ± SEM.

RESULTS

Rats which had received nicotine pressed more often ($p<0.01$) and avoided more shocks ($p<0.01$) than their saline trained partners (Table 1). There was a strain difference in performance, the cross-bred rats responding more frequently and taking fewer shocks than the Sprague-Dawleys, a difference which reached significance ($p<0.05$) only among the saline trained animals.

Substituting saline for nicotine caused a decrement in performance (see [6,10]). Since half the nicotine trained rats were tested with saline on the final day, performance was assessed not only for the final session but also for the previous 3 sessions when habitual treatment was given.

Adrenal Weight

Nicotine trained rats had heavier adrenal glands than their saline trained partners ($p<0.01$). The adrenal glands of cross-bred rats were heavier than those of the Sprague-Dawleys but the difference reached significance ($p<0.05$) only among those trained with nicotine.

Adrenal weight was found to be related to avoidance performance, rats which usually avoided most shocks having the heaviest adrenal glands (Fig. 1, Table 2). Figure

TABLE 1

THE EFFECTS OF STRAIN AND NICOTINE ON BEHAVIOUR AND ADRENAL WEIGHT

Strain*	No. of Rats	Treatment During Training	Adrenal Weight (mg)	Mean Pressing Total	Mean Shocks Avoided
S.D.	6	Saline	51 ± 2	145 ± 24	67 ± 9
C.B.	11	Saline	58 ± 2	227 ± 21	96 ± 5
S.D.	7	Nicotine	56 ± 2	369 ± 60	94 ± 9
C.B.	11	Nicotine	67 ± 3	413 ± 30	109 ± 2

*S.D. = Sprague-Dawley; C.B. = Cross-bred

Mean pressing and shock avoidance totals calculated for the three 60 min sessions preceding the final day of the experiment

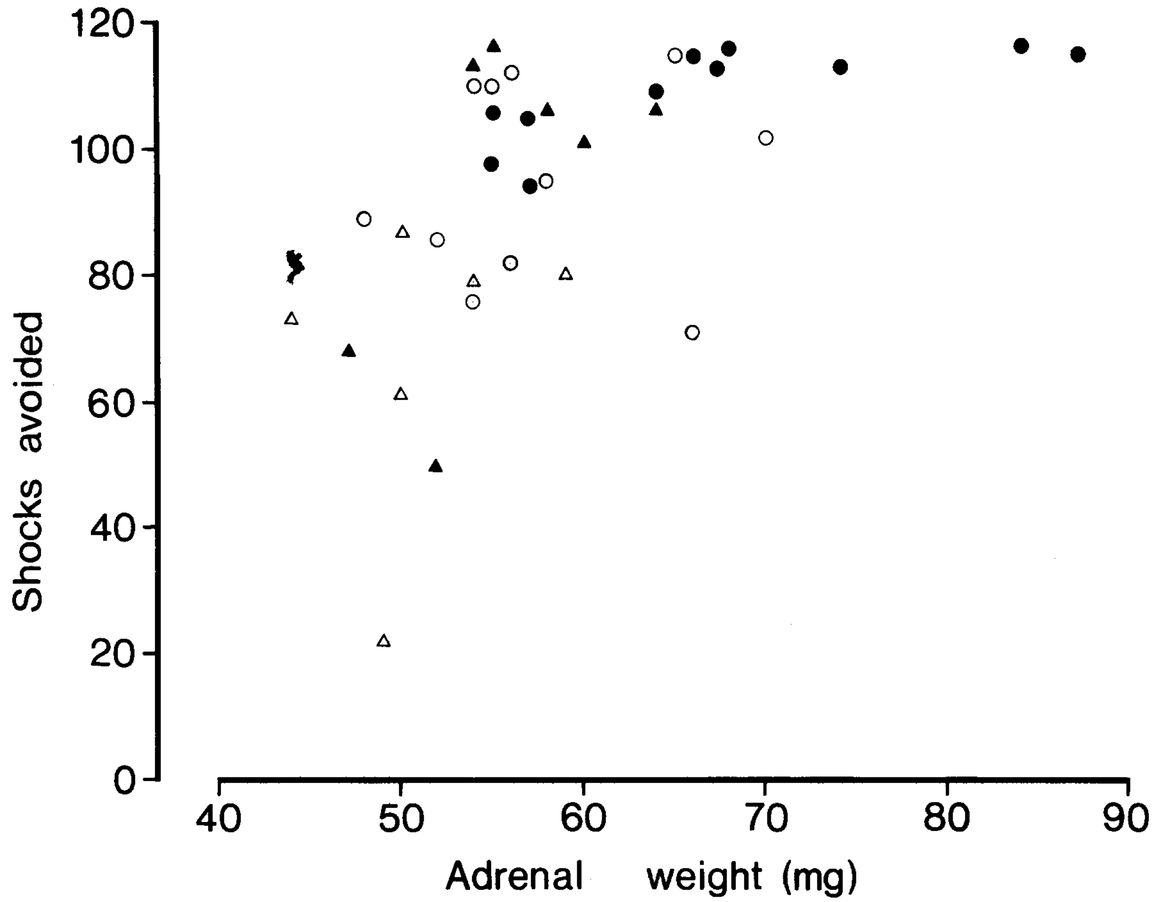

FIG. 1. Relationship between pooled adrenal weight and avoidance performance expressed as the mean number of shocks avoided (120, the maximum possible, less the number actually received) for the sessions preceding the final day. ○ Cross-bred; △ Sprague-Dawley. Closed points, rats trained and tested with nicotine; open points, rats trained and tested with saline.

TABLE 2

AVOIDANCE BEHAVIOUR, ADRENAL WEIGHT AND 5-HT, LINEAR CORRELATIONS*

		3 Sessions		Final Session	
	Adrenal Weight	Pressing Total	Shocks Avoided	Pressing Total	Shocks Avoided
Adrenal weight	–	0.49‡	0.56‡	0.44‡	0.39†
Hippocampal 5-HT, conc.	0.41†	0.29	0.34†	0.11	0.08
Hypothalamic 5-HT, conc.	–0.18	–0.24	–0.45‡	–0.33	–0.60‡

*N = 35
†$p<0.05$
‡$p<0.01$
Performance measured during the 3 sessions preceding the final day of the experiment and during the final session

TABLE 3

BODY WEIGHT, ADRENAL WEIGHT AND AVOIDANCE PERFORMANCE, LINEAR CORRELATIONS

Comparisons	Correlation Coefficients for All Rats
Body weight to adrenal weight	0.35*
$\frac{\text{Adrenal weight}}{\text{Body weight}}$ ratio to 3 day pressing	0.40*
$\frac{\text{Adrenal weight}}{\text{Body weight}}$ ratio to 3 day shocks avoided	0.39*
Body weight to 3 day pressing	0.09
Body weight to 3 day shocks avoided	0.14

*$p<0.05$

1 shows that the relationship between adrenal weight and shocks avoided was not in fact linear and the Spearman rank correlation coefficient, which does not assume linearity, was +0.64 ($p<0.01$). If the adrenal weight/body weight ratio was used, the correlations to performance were lower (Table 3).

If rats were treated with nicotine for 6 weeks without any behavioural training there was a small, but significant ($p<0.05$), increase in adrenal weight in both the Sprague-Dawley (means 39 ± 3 mg for the saline controls and 46 ± 2 mg for the nicotine treated rats) and cross-bred rats (saline 41 ± 2 mg, nicotine 45 ± 2 mg).

Corticosterone

Plasma corticosterone concentrations ranged from 15 to 28 μg/100 ml among the saline trained rats and from 10 to 35 μg/100 ml among the nicotine trained rats. There were no significant differences due to strain (mean for all Sprague-Dawley rats 19.4 ± 1.8 μg/100 ml, cross-bred rats 22.4 ± 1.1 μg/100 ml) or due to treatment, the mean plasma corticosterone concentrations being 20.6 ± 1.4 μg/100 ml for the saline rats, 22.9 ± 2.3 μg/100 ml for the nicotine rats and 20.2 ± 1.7 μg/100 ml for the rats trained with nicotine but tested on the last day with saline. Also there was no correlation between plasma corticosterone and the pressing rate or the number of shocks received by the rats.

5-Hydroxtryptamine

The nicotine trained rats had a slightly, though not significantly, higher hippocampal 5-HT concentration

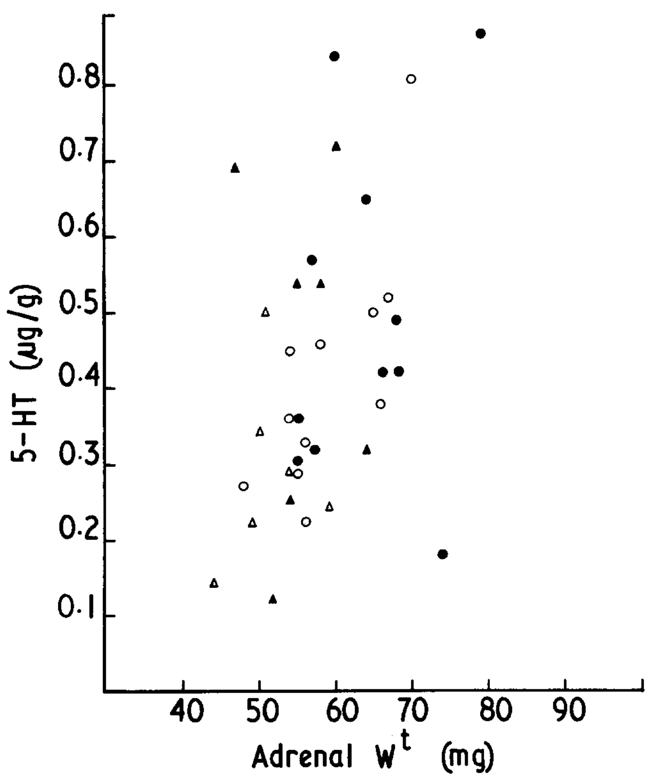

FIG. 2. Relationship between pooled adrenal weight and hippocampal 5-HT. ○ Cross-bred; △ Sprague-Dawley. Closed points, rats trained and tested with nicotine; open points, rats trained and tested with saline.

(mean 0.46 ± 0.04 μg/g) than the saline trained animals (mean 0.39 ± 0.04 μg/g). In addition the hippocampal 5-HT concentration was not affected if rats, trained with nicotine, were tested without nicotine (mean tested with nicotine 0.47 ± 0.07 μg/g, tested with saline 0.45 ± 0.07 μg/g). There was a low linear relationship between the hippocampal 5-HT concentration and the adrenal weight (Fig. 2, Table 3). Hippocampal 5-HT also correlated with the number of shocks usually avoided, but this correlation was not significant for avoidance performance during the final session (Table 3), when some of the nicotine trained rats were tested without nicotine and received many more shocks than usual.

There was a negative correlation between hypothalamic 5-HT and performance, particularly with the number of shocks avoided, the highest correlation being obtained with the performance during the final session (Table 3). Nicotine administration and withdrawal had no significant effect on hypothalamic 5-HT concentration (saline 0.84 ± 0.11 μg/g, nicotine trained and tested 0.82 ± 0.11 μg/g, nicotine trained but saline tested 0.90 ± 0.16 μg/g.

Noradrenaline

The mean NA concentration in the hippocampus was 0.66 μg/g and in the hypothalamus 1.38 μg/g. These two measures were related to each other ($r = +0.59$, $p<0.01$) but were unaffected by nicotine and were unrelated to any of our other parameters.

Other Relationships

A low correlation ($r = +0.35$, $p<0.05$) was obtained between body weight and adrenal weight (Table 3) but no correlation between age and body weight ($r = +0.14$). Body weight was unrelated to either measure of performance (Table 3). Adrenal weight was unrelated to the number of days required for training or the number of days subsequently on experiment. The severity of the decrement in performance when saline was substituted for nicotine was unrelated to any other measure.

Unavoidable Shocks

There were no significant differences among the high, low and unshocked rats in any measure. There was, however, a tendency for the plasma corticosterone to be higher among the shocked rats, the mean levels being 18 ± 5 μg/100 ml in the unshocked rats and 23 ± 3 and 29 ± 5 μg/100 ml in the low and high shock groups respectively.

DISCUSSION

The present results have shown that a good avoidance performance was associated with high adrenal weight and that nicotine improved performance and increased adrenal weight, data which suggest that a good avoidance performance may be associated with increased activity in the adrenal gland. The experiments were performed on two related strains of rats (Sprague-Dawley and Sprague-Dawley X Lister black hooded cross) because this gave a wide range of avoidance activity. It has been shown that nicotine administration to unstressed rats causes an increase in the plasma corticosterone concentration [2,8] and, therefore, it seems possible that the improved avoidance performance of the nicotine trained rats could be due, at least in part, to the effects which nicotine exerts on the pituitary-adrenal system. However the current studies did not demonstrate a correlation between avoidance performance and plasma corticosterone levels, but this may be due to the fact that corticosterone was measured only at 60 minutes in well-trained rats. Recent work, in which plasma corticosterone was estimated after 15 and 30 min avoidance sessions (C. F. Morrison unpublished results), has shown that, at 30 min, plasma corticosterone was higher in rats showing good avoidance performance.

Rats with heavy adrenals and which usually gave a good avoidance performance also had a high concentration of 5-HT in the hippocampus. These relationships with 5-HT were unaffected if, on the final day, the rats, notably those trained with nicotine but tested with saline, gave performances which differed from those they usually gave, suggesting that the hippocampal 5-HT concentration was not simply related to the number of shocks just received. This conclusion was confirmed by the results of the experiments in which the rats were not able to avoid the shocks. It has been reported that hippocampal 5-HT plays a role in the control of the secretion of adrenocorticotrophic hormone (ACTH) in unstressed rats [11] but it is not thought to be involved in the response to stress [12]. Thus the correlations between hippocampal 5-HT, adrenal weight and avoidance performance reported here could indicate that successful avoidance may be associated particularly with the pituitary-adrenal activity which comes under the control of serotonergic neurons in the hippocampus. Additional evidence for this hypothesis may be derived from the fact that nicotine improved performance and increased adrenal weight since previous results [2] have suggested that nicotine also may exert its effects on plasma corticosterone levels through the control mechanism for the unstressed situation.

Rats which were poor performers and received many shocks had elevated hypothalamic 5-HT. By contrast with the results with hippocampal 5-HT, hypothalamic 5-HT correlated best with the number of shocks just received. No explanation for this observation can be offered at present but the changes would not seem to be simply the result of the physical stress of receiving a large number of shocks since rats, subjected to unavoidable shocks, showed no increase in hypothalamic 5-HT. As found by Balfour *et al.* [2] nicotine did not affect NA levels in either hippocampus or hypothalamus, nor, apparently, did avoidance training.

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