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Central Catecholamine and Peripheral Noradrenaline Depletion: Effects on One-Way Trace-Conditioning

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OEI, T. P. S. AND M. G. KING. *Central catecholamine and peripheral noradrenaline depletion: effects on one-way trace-conditioning.* PHARMAC. BIOCHEM. BEHAV. 8(1) 25–29, 1978. – The present study evaluated the differential contributions of the central and peripheral catecholaminergic systems in aversive learning using trace-conditioning procedure. Twenty-seven male Wistar rats were randomly assigned to one of the three groups: central drug and peripheral saline (C_DP_S) injections, central saline and peripheral drug (C_SP_D) injections, and central saline and peripheral saline (C_SP_S) injections. Results showed that: (1) the rate of acquisition and the overall avoidance responses for the drug treated groups was significantly poorer than the control group; (2) there was no significant difference in performance between the two drug treated groups; and, (3) neither central nor peripheral catecholamine depletion had a significant effect on the secretion of plasma corticosterone. The findings failed to confirm the hypotheses (1) that central CA depletion is more detrimental than peripheral NA depletion, (2) that plasma 11-OHCS plays a mediating role in the acquisition of avoidance and escape responses in rats depleted of catecholaminergic systems.

Catecholamines Aversive learning Corticosterone 6-Hydroxydopamine

INVESTIGATION of the implication of peripheral NA in aversive learning, has consistently shown performance decrements on complex (two-way) but not on simple (one-way) avoidance learning tasks in rats injected peripherally with 6-hydroxydopamine (6–OHDA) [6, 8, 20, 21]. This was supported in part by Pappas and Sobrian [19] who found no difference between the performance of 6–OHDA injected and saline-injected rats on a one-way avoidance task. More recently, Lord, King and Pfister [16] and Oei [20,21] reported further supportive evidence in that depletion of peripheral sympathetic NA by 6–OHDA generally depressed two-way avoidance learning. These and other experimental findings, using either surgical sympathectomy [28] or immunosympathectomy [24, 25, 26], implicate the peripheral sympathetic secretion of NA in aversive learning although opinions differ as to the role it plays.

Studies involving central depletion of catecholamines (CAs) by 6–OHDA suggest that central CAs are also implicated in aversive learning (e.g. [2, 3, 20, 21]). When 6–OHDA was administered intraventricularly (IV) to rats [13], it was found that performance of a conditioned avoidance response, pole climbing, markedly decreased for the first two days postinjection, with most, but not all, animals recovering to pretreatment levels after a week. Rats chronically treated with IV injections of 6–OHDA (500 μg in several doses) required significantly more trials than controls to acquire an active avoidance response [22]. When injected intracisternally (IC) with 200 μg/rat of 6–OHDA or 6–OHDA in solution with other drugs (e.g., Pargyline or Desipramine), which results in a relatively selective depletion of brain NA and/or DA, rats failed to acquire a two-way shuttle avoidance response even with extended training in excess of one month [2,3]. In neonatal rats, injected subcutaneously at 7 days and tested at 60 days [23] or at 80 days [16] almost no acquisition of a two-way shuttle avoidance response was found. Avoidance decrements after depletion of NA and DA by administration of 6–OHDA were reversed [14,15] by both dopaminergic and noradrenergic agonists, e.g., l–DOPA and l–NA.

The literature therefore, indicates: (1) that both central and peripheral CAs are implicated in the acquisition of two-way shuttlebox avoidance responses, and (2) that central CAs may have a more pervasive effect than peripheral NA [20,21]. However, there is little direct evidence with which to evaluate the differential contributions of the central and peripheral CA systems in aversive

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learning. The aim of the present experiment was to evaluate the differential contributions of central and or peripheral CAs on the acquisition of one-way shuttle-box trace-conditioning avoidance task.

A one-way trace-conditioning task was chosen for the following reasons (1) to date, the effects of central CA depletion on this task have not been reported, (2) peripheral NA depletion has no effect on one-way delayed conditioning [20,21]. Since one-way trace-conditioning is not so readily acquired as delayed conditioning [1], it is possible that depletion of peripheral NA might produce significant decrements on the acquisition of the more difficult task.

Since ACTH and plasma corticosterone (11–OHCS) have also been implicated in the acquisition of avoidance responses [4, 7, 18] and since CAs, particularly central CAs may influence ACTH/plasma 11–OHCS secretion [5], the levels of plasma 11–OHCS were monitored.

METHOD

Animals

The animals were 27 naive male albino Wistar rats aged between 100–120 days at testing. The rats were housed individually in wire mesh cages (15.5 × 24 × 20 cm) with food and water freely available. The holding room was kept constant at 23 ± 1°C and a 12 hr light/dark cycle was maintained light off 1200–2400 hr).

Apparatus

An automated shuttle-box consisted of two compartments of clear Plexiglas with external dimensions of 60 × 125 × 12.5 cm. The two compartments were separated by a black metal guillotine door operated by an electric motor and each compartment had a clear Plexiglas lid. A Federal buzzer was attached externally to the end wall of each compartment. The grid floor was of stainless steel rods, 0.5 cm in diameter and spaced 1.2 cm apart. A microswitch was located beneath each grid on the common wall such that the transfer of mass as the rat crossed from one compartment to the other caused the depression of the corresponding microswitch. An automatic Sodeco printout timer recorded the latency between CS onset and the crossover response, accurate to 0.01 sec. All operations were controlled by programable logic circuits. The entire apparatus was situated in an air-conditioned room, with a constant temperature of 23 ± 1°C and an ambient illumination level of 2 log cd/m².

Procedure

Drug treatment. Two weeks before behavioral testing, animals were randomly allocated to the following groups: central saline and peripheral saline injections (C_SP_S), central drug and peripheral saline injections (C_DP_S) or central saline and peripheral drug injections (C_SP_D). Each rat in the C_D group was injected IC with 200 μg of 6–OHDA in 20 μl of 0.5% ascorbic acid and normal saline solution. The same amount of normal saline solution containing 0.5% ascorbic acid was administered to C_S animals. The P_D animals received a single intraperitoneal (IP) injection of 50 mg/kg body weight of 6–OHDA hydrochloride dissolved in normal saline solution with 0.5% ascorbic acid 8 hr before behavioral testing. The P_S animals received similar treatment to P_D rats, but without the drugs.

Behavioral procedure. Behavioral testing was carried out during the 4 hr following the middle of light cycle (0800–1200 hr) to standardize the diurnal variations effect on 11–OHCS levels obtained following the administration of shock [9].

Before behavioral testing, each rat was placed in the left compartment of the shuttle-box and allowed to explore the apparatus for 30 min with the guillotine door raised. The rat was then returned to the left-hand compartment if not already there and the programing equipment was switched on. Forty seconds later, the onset of the CS occurred producing an ambient noise level within the shuttle-box of approximately 90 db. The CS was presented for a duration of 1.5 sec or until the rat made the appropriate crossover response if this was less than 1.5 sec. Simultaneously with CS onset the guillotine door opened and if the rat had not crossed to the other side of the shuttle-box within 8 sec of CS onset, the US, an electric shock of approximately 2 mA, was applied to the grid floor in the compartment occupied by the animal. CS and US termination and closing of the door occurred when the animal crossed over to the grids to the opposite compartment. If the rat did not cross within 30 sec the CS and US were terminated, the guillotine door closed automatically and a new trial began. The intertrial (ITI) was 40 sec. Each rat was given 50 acquisition trials.

One animal from each group was tested in rotation. The apparatus was cleaned with hot water (80°C) after each animal and the tray under each grid was lined with clean paper toweling.

Plasma corticosterone assay. Immediately after an animal was sacrificed the blood from its cervical wound was collected in heparinized tubes and centrifuged at 4000 rpm for 15 min. The plasma was then collected, frozen, and stored for corticosterone determination. The maximum storage period was 2 weeks. The fluorometric method developed by Mattingly [17] was followed for an estimation of plasma 11–OHCS. All estimations were carried out in duplicate and blind with a recovery level of 98–101%.

Catecholamine assays. As soon as the blood was collected from the animal, the brain and the heart were removed, weighed and homogenized in 5 ml of 0.35 perchloric acid containing 100 mg/100 ml of sodium metabisulphate added just prior to use. Samples were centrifuged at 4000 rpm for 10 min. The supernatant was removed, the precipitate resuspended in another 5 ml of perchloric acid and the samples centrifuged again at 4000 rpm for 10 min. The supernatants were pooled and stored frozen if necessary (maximum period was 3 days) before absorption on a column of aluminia for CA estimations.

CAs were absorbed onto aluminia at Ph 8.5 then diluted with 1 N sulphuric acid and oxidized according to the method described by Haggendel [11] and Hinterburger [12]. After oxidation the samples were read for NA and DA at 400 and 300 nm for excitation and 520 and 374 nm for emission, respectively, on a fluorescence spectrophotometer.

RESULTS

The statistical analysis used to test all the behavioral measures was planned contrasts on repeated measures which is an extension of the technique of repeated measures trend analysis [10,27]. All comparisons were

TABLE 1

NULL HYPOTHESES FOR THE BEHAVIORAL MEASURES

Mathematical Statement of H_0	Description of comparison tested and its implications
$1H_0$: $2\mu C_SP_S - \mu C_SP_D - \mu C_DP_S = 0$	Differences in acquisition of responses between drug treated groups and control group combined over trial blocks.
$2H_0$: $\mu C_SP_D - \mu C_DP_S = 0$	Differences in acquisition of responses between peripheral drug and control treatments combined over trial blocks
$3H_0$: $T_{1-5} = 0$	The rate of change for all groups on the 5 trial blocks
$4H_0$: $1H_0 \times 3H_0 = 0$	Differences in the rate of change between drug treated and control groups on the 5 trial blocks
$5H_{0\ 2H_0} \times 3H_0 = 0$	Differences in the rate of change between peripheral and central drug treated groups on the 5 trial blocks

stated in the form of null hypotheses and tested with a Type 1 error rate of 0.05. As the hypotheses were not tested against any specific alternatives, no type II error rate was set. The coefficients to test for linear and quadratic components of trend over trial blocks were derived by the method proposed by Winer [27]. For biochemical measures Student's *t*-test was used.

On the basis of previous reported empirical findings [2, 3, 8, 20, 21] the following hypotheses were proposed (Table 1). Results of the planned contrasts for the several behavioral measures are summarzied in Table 2. The trends in the acquisition of avoidance responses are presented in Fig. 1 (right panel) and Fig. 1 (left panel) shows the mean response latency for treatment groups over trial blocks. As can be seen from Fig. 1 (both panels) the performance of drug treated groups (C_SP_D) and C_DP_S) was consistently poorer than the control (C_SP_S) group.

Results of the planned contrast analysis show that: (1) the overall avoidance responses and the response latency for the drug treated groups (C_SP_D and C_DP_S) was significantly lower than the control group (C_SP_S) (Fig. 1 and Table 2, 1 H_0); (2) the rate of acquisition for the drug treated groups was significantly slower than that of the control group ($4H_0$). However, when the performance of central drug treated and peripheral drug treated animals was compared there was no significant difference in the overall acquisition measures the rate of acquisition of avoidance responses and response latency between the two drug treated groups ($2H_0$, $5H_0$ and Fig. 1).

The means and standard errors of the escape latency for the three treatment groups are presented in Table 3. Planned contrasts analysis showed that the mean escape latencies of the drug treated groups were significantly lower than for the control group (Table 2, $1H_0$). There was no significant difference between C_SP_D and C_DP_S groups for the escape latency responses (Table 2, $2H_0$).

The means and SEs for the brain NA, DA, heart NA and plasma 11–OHCS are presented in Table 3. Independent Student *t*-tests were applied to the data for brain NA levels.

TABLE 2

SUMMARY OF THE PLANNED CONTRAST ANALYSIS AND THE DECISION RELATING TO H_0

Behavioural measures	H_0	*F* value*	Decision on *F*
(i) Mean Avoidance	1	12.618	reject
responses	2	0.450	accept
	3	12.390	reject
	4	0.037	accept
	5	0.037	accept
(ii) Mean response	1	9.182	accept
latency	2	0.217	accept
	3	72.414	reject
	4	1.973	accept
	5	1.248	accept
(iii) Grand Mean			
escape latency	1	4.959	reject
combined over	2	0.062	accept
trials			

*All tests were two-tailed with $p<0.05$ and $df = 1,24$.

It was found that the C_DP_S group was significantly different from the C_SP_D and C_SP_S groups ($p<0.01$), and there was no significant difference between C_SP_D and C_SP_S groups. Similarly for DA levels, the results of the *t*-test showed that only C_DP_S brain DA levels were significantly lower than those for the C_SP_D and C_SP_S groups ($p<0.01$). For heart NA levels, only the C_SP_D group levels were shown to be significantly lower than the C_SP_S and C_DP_S groups ($p<0.01$). There was no significant difference in plasma 11–OHCS levels for all groups tested.

DISCUSSION

Table 3 shows that there were substantial decrements in

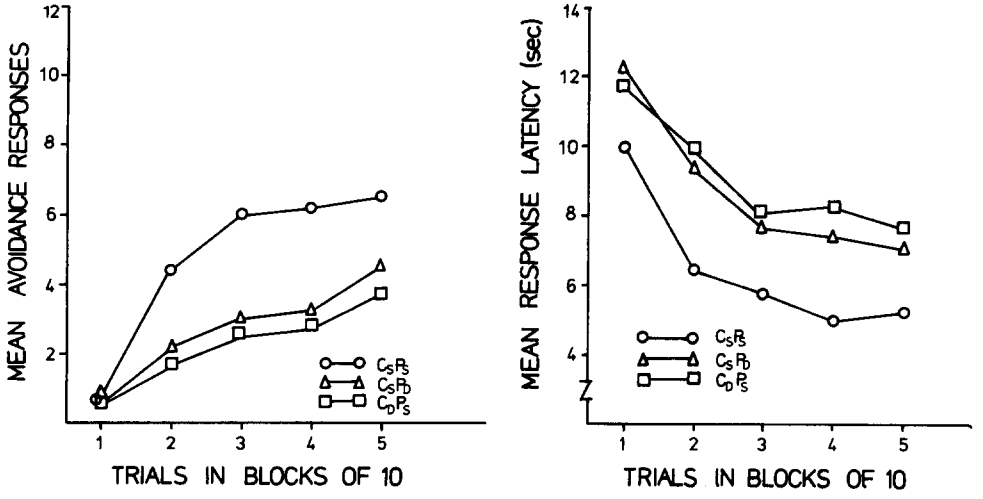

FIG. 1. Mean frequency and mean latency of responses over five trial blocks during acquisition of a one-way shuttle avoidance response using a trace-conditioning procedure.

TABLE 3

MEANS AND STANDARD ERRORS OF ESCAPE LATENCY AND BIOCHEMICAL LEVELS FOR THE CONTROL AND DRUG TREATED GROUPS

Treatment Groups	Escape Latency Mean ± S.E.	Heart NA Mean ± S.E.	Whole Brain DA Mean ± S.E.	Whole Brain NA Mean ± S.E.	Plasma 11-OHCS Mean ± S.E.
C_SP_S	9.15 ± 0.13	732.4 ± 43.8	644.4 ± 49.0	447.2 ± 18.0	39.6 ± 2.1
C_SP_D	10.21 ± 0.57	126.7 ± 15.6	612.8 ± 24.8	397.3 ± 29.9	41.1 ± 2.6
C_DP_S	10.36 ± 0.43	703.1 ± 35.4	142.2 ± 20.0	54.3 ± 1.9	39.5 ± 1.7

whole brain NA (to 12% of control levels) and DA (to 22% of control levels). The main effects for Central NA depletion and for Central DA depletion were each highly significant which is consistent with the results of previous findings [2, 3, 14, 15, 20, 21]. In the non-drug (C_SP_S) group, the effect of training on one-way trace-conditioning was to raise the levels of central DA significantly (122% of untrained control levels) but not central NA (101% of untrained control levels), indicating that in the non-drug group, whole brain DA was more elevated by trace-conditioning. However, the results of Oei and King [20,21] one-way and two-way avoidance with delayed conditioning show that brain NA levels were more elevated by training. The reversal in the levels of NA and DA in Oei and King [20,21] on the one hand and in the present study on the other would thus seem to be a function of the classical conditioning parameters: Trace-conditioning may elevate DA more and delayed conditioning may elevate NA more.

Changes in central NA and DA levels for untrained animals in the centrally drug treated groups were respectively from 90.1 [21] to 54.3 ng/g tissue and from 118.3 to 142.2 ng/g tissue. Thus it would appear that central DA was more elevated by training in the centrally treated animals. These results are consistent with the results of Oei and his associates [20,21].

The results of the present study also showed that IP injection of 6–OHDA produced a significant reduction of heart NA (to 17% control). This suggests that peripheral NA is significantly depleted by injection of 6–OHDA. The percentage of reduction for IP injection is of the same order as that for IC injection with both methods of injection reducing CA levels by almost 80% of the appropriate controls [20,21].

The statistical results for plasma 11–OHCS showed that neither peripheral NA nor central CA depletion had a sinificant effect on the secretion of plasma 11–OHCS after 50 trials of trace conditioned avoidance learning. The present results and results of Oei and King [20,21] have failed to demonstrate a mediating role of plasma 11–OHCS in the acquisition of avoidance and escape responses. Since peripheral NA and central CA were still depleted by approximately 80% of control levels at the end of training, it is reasonable to suggest that the observed retardation in acquisition of escape and avoidance responses in one-way learning using the trace-conditioning procedure is strongly influenced by depletion of either central or peripheral CAs.

One result of the present study, that IP injection of 6–OHDA led to significantly poorer escape and avoidance performance when compared with saline injected rats, is consistent with the finding of Di Giusto and King [8] using this conditioning procedure. Oei and King [20,21] showed no significant difference in escape and avoidance in one-way shuttle box responses using the delayed conditioning procedure between the 6–OHDA and saline injected

rats. By contrast, the present study using a trace conditioning procedure showed a significant difference in the acquisition of escape and avoidance performance between the drug and saline injected control. The main procedural difference between the present study and that of Oei and King ([21], Experiment 3) was that trace-conditioning was used in the present study whereas delayed conditioning was used in that study. Trace-conditioning has been shown to be a more difficult task for the rat to learn than delayed conditioning [1,8] and performance of the present non-drug (C_SP_S) groups lend further support to that finding. In delayed conditioning [20,21], the non-drug rats performed 85% avoidance responses by the end of 40 trials whereas with trace-conditioning the C_SP_S animals gave only 62% avoidance responses after the same number of trials. The results of the present study and the above-mentioned studies on peripheral NA depletion thus indicate that increase in task difficulty leads to significant decrements in escape and avoidance responding by NA depleted animals.

The results of the present study showing that animals depleted of central CAs performed significantly poorer in escape and avoidance were consistent with previous findings (e.g. [2, 3, 14, 15, 20, 21]). The present finding adds to the growing list of effects of central CA depletion on animal learning.

Table 2 ($2H_o$) showed that there was no significant difference in the escape and avoidance performance between peripheral NA and central CA depleted animals. This finding does not support the hypothesis that central CA depletion is more detrimental than peripheral NA depletion assuming that the depletion of central CA and peripheral NA are of the same order. For the hypothesis to be supported, a significant difference in the escape and/or avoidance responses should have resulted. It must be noted, however, that the present results were based on 50 avoidance trials in one session. It is possible that the non-significant result obtained in the present study between central and peripheral CA depletion was due to the fact that not enough training trials were given to the animals. Given more training trials significant differences in performance between central CA and peripheral depleted animals might emerge. Similar arguments can also be put forward to explain the retardation of avoidance performance observed in previous mentioned studies for the central and peripheral CA depleted animals.

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