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6-Hydroxydopamine Induced Catecholamine Depletion and Passive Avoidance Learning in Rats

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OEI, T. P. S. AND C. P. NG. *6-Hydroxydopamine induced catecholamine depletion and passive avoidance learning in rats.* PHARMAC. BIOCHEM. BEHAV. 8(5) 553–556, 1978. – Rats were injected with 6-hydroxydopamine either intracisternally, intraperitoneally, or both, in order to examine the effects of central vs. peripheral catecholamine depletion on a step-down passive avoidance task. All rats acquired the response at the end of the five acquisition trials but the rates of acquisition of the drug-treated groups were significantly different from the control group. No significant difference in the performance results were observed between groups during the extinction period. These findings failed to confirm the hypothesis that an intact central and/or peripheral catecholaminergic systems may be necessary for the acquisition and extinction of a step-down passive avoidance response. In addition, this study also showed that plasma corticosterone levels in the rats depleted of central or peripheral catecholamine did not differ significantly from each other after passive avoidance training.

6-OHDA Passive avoidance Catecholamines 11-OHCS

6-HYDROXYDOPAMINE (6-OHDA) has been shown to produce long-term and specific depletion of peripheral catecholamines (CAs) and of central CAs at appropriate doses (e.g. [10, 14, 20]). Many studies have taken advantage of this unique property of 6-OHDA for examination of the effects of depletion of peripheral and/or central CAs in rats on avoidance performance (e.g. [4, 8, 11, 12, 13, 15, 16, 17, 18]). In these studies, active avoidance has been the required behavioural response. While the concept of the conditioned fear response in most psychophysiologically oriented theories (e.g. two-process theory) has been largely derived from experiments on active avoidance, classically conditioned fear has been said to motivate the performance of passive avoidance responses as well [1,3]. However, there are few experiments in the current literature which examine the effect of peripheral and/or central CA depletion on passive avoidance responses. It has recently been shown that acute depletion of peripheral noradrenaline (NA) by 6-OHDA retards the acquisition of a step-down passive avoidance response [8]. However, it has also been found that depletion of central CAs by 200 μg of 6-OHDA does not alter significantly the acquisition of a step-through passive avoidance response [4].

The present experiment was designed to clarify the roles of peripheral and central CA containing neurons in the passive avoidance response. Besides the possible involvement of central and/or peripheral CAs in the acquisition of passive avoidance, there is substantial evidence in the literature to suggest the involvement of adrenal corticosteroids (and presumably adrenocorticotropic hormone (ACTH)) in both the acquisition and the retention of a passive avoidance response [2, 5, 6, 7, 9, 15]. For this reason, circulating plasma 11-hydroxycorticosterone (11-OHCS) levels of rats were also measured in the present study.

METHOD

Animals

Thirty-two naive male Wistar rats aged between 100–120 days at the time of testing were used. Rats were randomly allocated to one of the following groups of eight animals each: central drug and peripheral drug injections (C_DP_D), central drug and peripheral saline injections (C_DP_S), central saline and peripheral drug injections (C_SP_D) and central saline and peripheral saline injections (C_SP_S). Rats were housed individually in stainless steel cages (15.5 × 24 × 20 cm) with food and tap water freely available. The holding room was kept at 23 ± 1°C and 12 hr dark/light cycle was maintained (light off 1200–2400 hr).

Apparatus

The apparatus consisted of a clear Plexiglas box with internal dimensions of 27.5 x 35 x 45 cm in depth to the grid floor. The top of the box was open and the walls were covered externally with opaque white adhesive wall paper. The grid floor consisted of parallel stainless steel rods, 6 mm in dia. and set 2 cm apart, centre to centre. Eight and one half cm below the grid floor was a stainless steel tray covered with paper towel. In one corner of the box, 41 cm from the top, a Plexiglas platform was attached, 16 x 15.5 cm, and covered with white tape to prevent the rats from slipping when placed on it. This platform was balanced on a sensitive microswitch which could be tripped by the weight of the animal. The microswitch was wired to programing equipment which consisted of a programable scheduler, a Sedeco print-out timer and a shock generator delivering a constant current of approximately 1 mA. The apparatus was housed in an air-conditioned cubicle in which the temperature was maintained at 23 ± 1°C.

The apparatus for the collection of blood, removal of brains and biochemical assays has been described previously [15].

Drug Treatment

The drug treatment was the same as described previously [15]. Briefly, the C_D rats were injected intracisternally (IC) with 200 μg of 6-OHDA in 20 μl of saline solution containing 0.5% of ascorbic acid. Fourteen days after IC treatment and 8 hr before behavioural testing, the P_D rats received an intraperitoneal (IP) injection of 50 mg/kg of the drug solution containing 0.5% of ascorbic acid. Rats in the C_S and the P_S groups received the vehicle solution without the drug at the same time when C_D and P_D animals received their injections.

Procedure

Behavioural testing was carried out during the last quarter of the light cycle (0800–1200 hr). This was done two weeks after the rats had their central drug treatments and 8 hr after IP injection. All rats were given five acquisition trials of passive avoidance, one trial per day, each trial of 180 sec duration. One hundred-eighty sec was chosen because it has been shown to be the optimal time for the acquisition of passive avoidance [8]. At the start of each trial, the rat was removed from the home cage and placed on the platform. This triggered the latency recorder of the chronoscope. When the animal stepped down the platform onto the floor, the latency recorder stopped immediately, and thus the latency of response was recorded. The rat received approximately 1 mA of electric shock to the feet for a maximum period of 8 sec if it did not climb back to the platform during the shock period. If it climbed back on the platform during the shock period, the shock automatically terminated. Sixty sec after the completion of a trial, the rat was returned to its home cage. The passive avoidance box was cleaned after each rat with hot water and a fresh paper towel was placed on the steel tray below the box.

Extinction testing started the day after acquisition sessions ended and consisted of five trials with one trial per day. Each trial lasted for 300 sec. As in the acquisition session, placing the rat on the platform of the passive avoidance box triggered the latency recorder and marked the beginning of the trial. Unlike acquisition trials, no electric shock was delivered to the grid floor during extinction trials when the rat stepped down from the platform onto the grid floor. Sixty seconds after the completion of an extinction trial, the rat was returned to its home cage. The passive avoidance box was cleaned with hot water after each animal and the paper towelling replaced.

Biochemical procedure. Each rat was removed from the passive avoidance box and decapitated by guillotining immediately after its last extinction trial. The same procedure as previously described [15] was followed for the collection of blood for plasma 11-OHCS assay and rapid removal of the brain and heart for CAs and NA assay respectively.

RESULTS

Acquisition and Extinction of Passive Avoidance Responses

For the purpose of statistical analysis the response latencies of each rat was used. The means and SE of response latency for the four treatment groups in the acquisition and extinction of the passive avoidance task is presented in Table 1. A three-way ANOVA with repeated measures on one variable [22] was applied to the latency data for acquisition trials to test for the main effects of Central CA depletion, Peripheral NA depletion and time over Days. The results of the analysis revealed no significant differences for the main effects of central CA and of peripheral NA depletion, meaning that overall acquisition of the response was not significantly affected by depletion of either central CAs or peripheral NA. However, there was a significant Days effect, $F(4,112) = 51.543$, $p<0.01$, and interaction of the Central x Peripheral x Days effect, $F(4,112) = 3.687$, $p<0.01$. These significant results indicate that the overall rate of acquisition for all treatment groups improved across days and that the rate of improvement in some treatment groups differed from others. Post hoc Scheffé tests [22] revealed that the rate of acquisition for the C_DP_D group was the fastest ($p<0.05$) and the C_SP_D and C_DP_S groups lowest ($p<0.05$) with the control group intermediate. The analysis also revealed that there was no difference between the rate of acquisition for the C_SP_D and C_DP_S groups.

A three-way ANOVA with repeated measures was applied to the extinction data. The analysis revealed no significant main effects except the Days factor, $F(4,112) = 4.001$, $p<0.01$, and no significant interaction effects. The results thus indicate that neither central nor peripheral CA depletion by 6-OHDA has significant effect on the extinction of a step-down passive avoidance response. All groups, having acquired the response to the same level, extinguish at the same rate during the early stages (Days 1–5) of extinction.

Plasma 11-OHCS. The means and SE for the plasma 11-OHCS levels of the four treatment groups are presented in Table 2. A two-way ANOVA [22] was applied to the data and the results of the analysis revealed no significant main effects for Central or Peripheral depletion suggesting that depletion of central or peripheral CAs by 6-OHDA produces no significant difference in the levels of plasma 11-OHCS secretion in rats after 10 days of step-down passive avoidance training.

Whole brain NA and DA. The means and SE of the whole brain dopamine (DA) and NA levels (ng/g tissue) for the four treatment groups can be seen in Table 2. A

TABLE 1

MEANS AND STANDARD ERRORS OF LATINCY OF RESPONSES IN SEC ON TEN SUCCESSIVE DAYS DURING FIVE ACQUISITION AND FIVE EXTINCTION OF A STEP-DOWN PASSIVE AVOIDANCE RESPONSE

Treatment* Groups	Days 1	2	3	4	5
Acquisition					
C_SP_S	20.2 ± 10.7	145.2 ± 23.9	132.5 ± 23.8	147.9 ± 20.5	174.4 ± 5.6
C_SP_D	21.7 ± 8.2	94.6 ± 32.2	158.1 ± 21.9	158.1 ± 21.9	158.1 ± 22.3
C_DP_S	63.5 ± 24.5	100.7 ± 30.3	157.6 ± 22.3	167.4 ± 22.8	168.1 ± 23.1
C_DP_D	57.1 ± 26.9	175.2 ± 10.5	176.2 ± 4.9	178.3 ± 32.0	177.2 ± 3.2
Extinction					
C_SP_S	281.7 ± 14.4	227.3 ± 32.1	259.1 ± 36.7	210.1 ± 35.3	222.5 ± 40.4
C_SP_D	261.6 ± 37.1	255.3 ± 36.9	228.2 ± 47.1	226.4 ± 48.2	226.3 ± 48.2
C_DP_S	262.8 ± 37.3	262.8 ± 37.1	263.4 ± 36.9	247.1 ± 36.3	204.2 ± 46.1
C_DP_D	272.1 ± 24.8	268.7 ± 31.3	242.5 ± 34.4	273.3 ± 30.4	253.3 ± 33.9

* See text for abbreviations

TABLE 2

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

Treatment* Groups	Whole brain (ng/g tissue) NA Mean ± SE	DA Mean ± SE	Plasma 11-OHCS (μg/100ml) Mean ± SE	Heart NA Mean ± SE
C_SP_S	475.7 ± 55.2	782.8 ± 37.3	20.0 ± 2.78	759.9 ± 50.9
C_SP_D	483.9 ± 51.8	708.3 ± 57.6	708 ± 57.6	269.3 ± 20.20
C_DP_S	176.3 ± 24.8	163.7 ± 18.5	22.4 ± 2.79	690.3 ± 42.5
C_DP_D	173.0 ± 22.3	198.6 ± 21.8	21.1 ± 2.15	254.4 ± 30.5

* See text for abbreviations

two-way ANOVA was applied to the brain NA and DA data, and the analysis showed that only central administration of 6-OHDA caused depletion of NA, $F(1,28) = 54.443$, $p<0.01$, and DA, $F(12,8) = 261.9$, $p<0.01$.

Peripheral heart NA. The means and SE of peripheral NA are presented in Table 2. A two-way ANOVA revelaed that only the peripheral main effects were significant, $F(1,28) = 257.3$, $p<0.01$.

DISCUSSION

Analysis of five days data revealed that both central drug treated and saline treated groups learn the acquisition of a step-down passive avoidance response. Table 2 clearly shows that the C_D groups performed almost as well as the C_S groups after Day 2 of the testing, which replicates previous findings [4].

Although both central drug treated and saline treated rats acquire the step-down passive avoidance response, the rate of acquisition for the central drug (C_D) groups was significantly slower than the control (C_S) groups. The slower rate of acquisition, as indexed by significant trials by groups effects for the C_D and C_S groups, provides some direct evidence that the results obtained in the present study and other studies [11, 12, 13, 15, 16, 17, 18] are due to the effect of CA depletion rather than locomotor impairment or 6-OHDA treatment induced physical weakness. If the drug had produced significant locomotor impairment or physiological debilitation, an apparent enhancement of passive avoidance performance should have occurred. This is because the passive avoidance response requires an animal not to move in order to avoid foot shock.

The effects of IP injections of 6-OHDA on the rate of acquisition of step-down passive avoidance is consistent with the findings of others [8,21]. The present study thus shows that depletion of either peripheral or central CAs retards the rate of acquisition of a step-down avoidance response but depletion of those amines does not prevent the animals from eventually learning the task.

The fact that the rate of acquisition of a step-down passive avoidance response for the C_DP_D groups was significantly faster than the other three groups, is at variance with the results obtained in previous active

avoidance studies where the experimental design was essentially the same as that of the present study. In those studies [15,18] the C_DP_D group regularly showed poorer acquisition of active avoidance responses than the three other groups. Although the results are empirically opposite this outcome would be expected on theoretical grounds [19]. It is suggested that the increase in the rate of acquisition in the C_DP_D group is due to the double drug treatment interfering with the conditioning of the response, or the level of motivational state. If the conditioning or the level of motivational state of the C_DP_D animals are impaired, the rats would be expected to perform worse in active but better in passive avoidance performance.

The present results concerning extinction of a passive avoidance response show that drug treated rats extinguish at about the same rate as the control animals. The study shows that the acquisition and extinction of a step-down passive avoidance task is still possible after IC and IP injections of 6-OHDA which depletes central NA to 36% and central DA to 25% and peripheral heart NA to 28% of control levels. The finding is consistant with other findings in the literature which show that peripheral noradrenergic neurons and possibly central CA neurons apparently function normally even with a depletion of over 50% of their CA stores [10].

That plasma 11-OHCS levels do not seem to play a mediation role confirms previous studies which have used the active avoidance task [15, 16, 17, 18]. This is borne out by the fact that there was no significant change in performance obtained in either IC or IP drug treated rats. Thus, if 11-OHCS levels do have a mediation role in 6-OHDA treated rats it can only occur during the course of aversive training [5].

In summary, this study provides no firm evidence in support of the notion that central and/or peripheral CAs are involved in the overall acquisition and extinction of a step-down passive avoidance response. However, the rate of acquisition was significantly affected. In conclusion, the findings suggest that an intact central and/or peripheral CA systems may not be necessary for the acquisition and extinction of a step-down passive avoidance response, although central CA systems had been shown to be important and necessary for the acquisition of active avoidance responses [4, 11, 12, 15, 16, 18].

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