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Effects of Extended Training on Rats Depleted of Central and/or Peripheral Catecholamines

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OEI, T. P. S. AND M. G. KING. *Effects of extended training on rats depleted of central and/or peripheral catecholamines.* PHARMAC. BIOCHEM. BEHAV. 9(2) 243–247, 1978.—Thirty-two Wistar rats were used to investigate the effects of extended training on avoidance performance of rats depleted of Catecholamines. They were injected with 6-hydroxydopamine either (i) intracisternally, (ii) intraperitoneally, or (iii) both IP and IC, and trained on a two-way shuttlebox avoidance task. The results on peripheral noradrenaline depletion led to the conclusion that extended training ameliorates the observed behavioural deficit significantly back to the level of controls. The conclusion that is indicated by the present central catecholamine depletion are: (i) central catecholamine depletion has long term effect on behavioural deficit, (ii) central depletion is more detrimental to avoidance learning than peripheral depletion, and (iii) plasma corticosterone plays no significant mediating roles.

Avoidance Brain Catecholamines Corticosterone 6-Hydroxydopamine Training

PREVIOUS findings on peripheral noradrenaline (NA) depletion using 6-hydroxydopamine (6–OHDA) indicated that peripheral NA is implicated in the acquisition of two-way avoidance using simultaneous conditioning and in the acquisition of one-way avoidance using trace-conditioning but not in one-way delayed conditioning avoidance responses [3, 10, 13, 14]. These findings show that rats depleted of peripheral NA perform as well as their controls on some aversive tasks, but not on others which raise the question of the nature of the retardation in acquisition of the avoidance tasks. It is possible that the significant retardation observed was due to insufficient training implying [12,13] that if experimental animals were given extended training trials they would learn their avoidance tasks. There is no reliable experimental evidence reported to date to show that training helps improve impaired learning in rats depleted of peripheral NA. It is therefore hypothesized that given extended training, rats depleted of peripheral NA may eventually perform as well as controls in the acquisition of two-way active avoidance with simultaneous conditioning and one-way trace-conditioning avoidance [12,13].

Evidence of the effect of extended training on deficits in the avoidance performance of rats depleted of central CAs is inconclusive. Laverty and Taylor [7] showed that a pole-climbing conditioned avoidance response was significantly retarded for the first two days but returned to pre-treatment levels 6 days later. Cooper, Breese, Grant and Howard [1] demonstrated that rats depleted of central CAs completely failed to acquire a two-way shuttlebox avoidance response even after 75 training days. The finding of Cooper *et al.* [1] were also confirmed by Lenard and Beer [8,9] on a shelf-jump avoidance task.

It has been argued that plasma 11–OHCS levels may play a mediating role in avoidance performance of rats depleted of central and peripheral catechaminergic systems [2, 4, 12, 13, 14]. Therefore, plasma 11–OHCS levels following acquisition were monitored.

The aim of the present study was to investigate the effects of extended training on central CA and peripheral NA depleted rats in the acquisition of two-way avoidance response.

METHOD

Animals

Thirty-two naive male Wistar rats aged 100–120 days at the time of testing were used. The rats were housed individually in wire mesh cages (15.5×24×20 cm) in a room with temperature thermostatically controlled at 23 ± 1°C. Rats had ad lib access to food and water. A 12 hr light/dark cycle was maintained (light off 1200–2400 hr).

Apparatus

The shuttlebox apparatus used was the same as that described in detail previously by Oei and King [12]. Briefly, the

apparatus consisted of an automated shuttlebox, and Sodeco print out, a programmable logical circuit and two shock generators. The apparatus for collection of blood, removal of brains and biochemical assays was the same as described previously [12].

Procedure

Drug treatment and behavioral procedures. The drug treatment procedure was the same as that described in detail by Oei and King [12]. Briefly, the animals were randomly allocated to one of the central saline and peripheral saline injections (C_SP_S), central saline peripheral drug injections (C_SP_D), central drug and peripheral saline injections (C_DP_S) and central drug and peripheral drug injections (C_DP_D) groups. The C_D animals were intracisternally injected with 200 μg of 6-OHDA in 20 μl of saline solution containing 0.5% of ascrobic acid and the P_D rats injected intraperitoneally with 50 mg/kg of the same drug 14 days after C_D injection and 8 hr before behavioral testing. The control rats for C_S and P_S groups received similar treatments at the same time as the C_D and P_D groups without the drug.

The general behavioral procedures were the same as described by Oei and King ([12], Experiment 2) except that each animal was given one such testing session daily for 10 days. Testing was carried out between 0800–1200 hr and each testing session consisted of 35 acquisition trials of two-way avoidance learning with simultaneous conditioning using a 40 sec ITI. The CS was an ambient noise level within the shuttlebox of approximately 60 db. The US was an electric shock of approximately 2 mA and the CS–US interval was 8 sec. On Day 1, rats were given a 30 min exploratory period but not on subsequent days.

One animal from each group was tested in rotation. The apparatus was cleaned with hot tap water and dried after each animal and the tray under each grid was lined with clean paper toweling.

Biochemical Procedure

Plasma corticosterone assay. Immediately after an animal was sacrified 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 [11] 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.

Catecholamines were absorbed onto aluminia at Ph 8.5 then diluted with 1 N sulphuric acid and oxidized according to the method described by Haggendel [5] and Hinterburger [6]. After oxidation the samples were read for NA and DA at 400 and 300 nm for exitation and 520 and 374 nm for emission, respectively, on a fluorescence spectrophotometer.

RESULTS

The following behavioral measures were obtained from the performance of each rat for the purpose of analysis in the present experiment: mean number of avoidance responses over 10 days, mean response latency over 10 days, mean escape latency, and mean number of escape trials before the first avoidance.

In addition to the above behavioral measures, four biochemical levels were obtained from each rat for the analysis. They were: plasma 11-OHCS level, whole brain NA level, whole brain DA level, and heart NA level.

A three-way ANOVA with repeated measures on the days factor was used to analyze each of the behavioral measures and a two-way ANOVA was applied to each of the biochemical levels.

A. Analysis of the Mean Number of Avoidance Responses Over 10 Days

The mean number of avoidance responses over the 10 days was presented in Fig. 1. The results of the ANOVA showed that central CA depletion effect (summed over all days) was significant, $F(1,28)=16.071$, $p<0.001$, indicating that the overall performance of the avoidance response by central CA depleted animals is significantly poorer than the control groups. There was no overall performance difference in the avoidance responses between the peripheral NA depleted groups and their control groups. The main effects of Days, $F(9,252)=18.988$, $p<0.001$, and the Days×Central CA depletion effect, $F(9,252)=4.977$, $p<0.01$, were significant suggesting that while avoidance performance across treatment groups improved significantly during successive days, not all groups improved at the same rate. The rate of improvement for the central CA depleted (but not peripheral NA depleted) rats was significantly depressed compared to that of the control groups. As can be seen from Fig. 1 the mean acquisition of two-way shuttlebox avoidance responses over 10 days for the C_DP_D and C_DP_S groups was consistently lower than that of C_SP_S and C_SP_D groups. There was an increase in the mean acquisition responses for C_DP_S rats on Days 2 and 3 with the highest mean of avoidance responses ($\bar{X}=13.5$) on Day 9, but dependent t tests showed that this increased response was not significant. The highest mean acquisition responses ($\bar{X}=9$) for the C_DP_D group was at Day 6. Again, dependent t test showed that there was no significant differences in mean acquisition responses on any day.

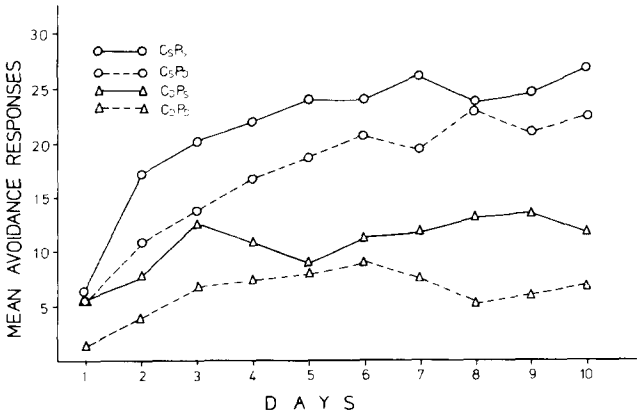

FIG. 1. Mean frequency of avoidance responses over ten days of extended training on a two-way shuttle avoidance response.

B. Analysis of Mean Response Latency Over 10 Days

As in Measure (A) above, the mean response latency over the 10 days was presented in Fig. 2. The statistical results obtained were similar to those obtained from measure (A) above, i.e., only the central CA depletion, $F(1,28)=18.771$, $p<0.001$, Days $F(9,252)=5.514$, $p<0.001$, and Central Depletion×Days, $F(9,242)=5.514$, $p<0.001$, effects were significant. The significant central CA depletion effect indicates that summed over 10 days, response latencies for rats depleted of central CAs were significantly lower than that for the C_S control groups. The significant Days effect and significant Central depletion×Days effect suggest that while response latencies summed across groups decreased during testing on successive days, not all groups improved at the same rate. In fact, the rate of improvement over 10 days for the central CA depleted, but not for the peripheral NA depleted, rats was significantly depressed when compared to the control groups. Figure 2 shows that the mean response latencies of the C_DP_S and C_DP_D animals are much higher than that for the C_SP_S and C_SP_D groups. The largest difference in mean response latencies between the central CA depleted (C_D, $\bar{X}=9.2$ sec) and control (C_S, $\bar{X}=5.8$ sec) groups occurred on Day 8.

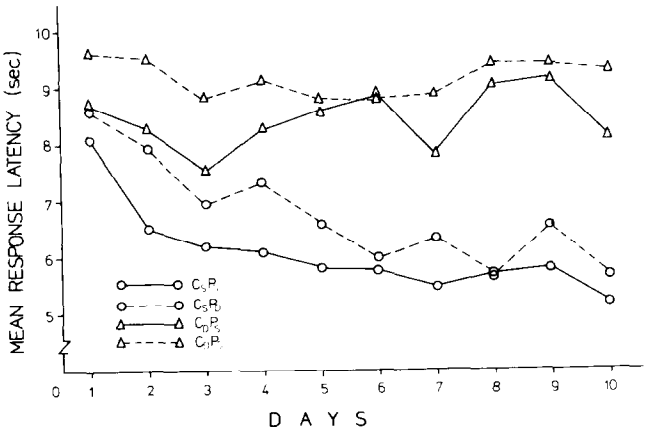

FIG. 2. Mean latency of responses over ten days of extended training on a two-way shuttle avoidance response.

C. Analysis of the Mean Number of Escape Responses Before the First Avoidance Over 10 Days

This measure was obtained by adding up the number of escape responses of each rat before the first avoidance response and the mean used for the analysis. A three-way ANOVA results showed that only the three main effects were significant: for Central CA depletion, $F(1,28)=13.383$, $p<0.001$, for Peripheral NA depletion, $F(1,28)=13.530$, $p<0.001$, and for the Days effect, $F(9,252)=5.645$, $p<0.001$. The significant main effects for Central and for Peripheral depletions indicate that rats injected with 6-OHDA either peripherally or centrally required more trials before making the first avoidance response. The significant main effect for Days suggests that on the whole there was a general decrease in the number of trials required to make the first avoidance response (Fig. 3). As can be seen in Fig. 3 the mean number of escape trials before the first avoidance response for the C_SP_D and C_DP_S groups, declined to about the same level as

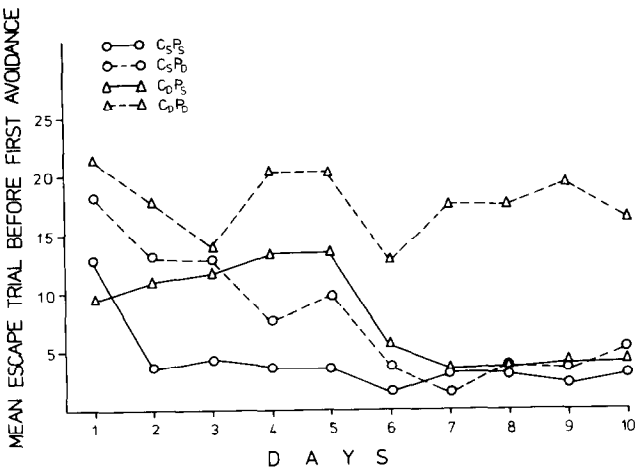

FIG. 3. Mean frequency of escape trials before the first avoidance response over 10 days of extended training on a two-way shuttle avoidance response.

the C_SP_S group on Day 6 and remained thus for the rest of the experimental period of 10 days. The mean number of escape trials before the first avoidance response for the C_DP_D group remained significantly high for the period of 10 days.

D. Analysis of the Mean Escape Latency Over 10 Days

This measure was obtained by adding the escape latency of each rat within the daily 35 acquisition trials. A three-way ANOVA with repeated measures showed that only the main effect of central CA depletion was significant, $F(1,28)=9.394$, $p<0.01$, suggesting that animals depleted of central CAs took longer to escape than control animals. The lack of a significant days effect suggests that there was no improvement in escape speed across days for all the groups tested.

E. Plasma 11-OHCS Analysis

The means and SEs of the plasma 11-OHCS levels after 10 days of training for the four treatment groups are presented in Table 1. An Analysis of Variance was applied to the mean plasma 11-OHCS levels and no significant effect was found.

F. and G. Whole Brain NA and DA Analysis

The means and SEs of the whole brain NA and DA for the four treatment groups can be seen in Table 1. A 2×2 ANOVA was applied to the data for whole brain NA. The results showed that only the main effect of central NA depletion was significant, $F(1,28)=109.874$, $p<0.001$, indicating that injection of 200 μg/20 μl of 6-PHDA into the cisternum magnum produces significant reduction in whole brain NA levels (33% of control). Similarly, a 2×2 ANOVA was applied to whole brain DA levels. The results showed that 200 μg/20 μl of 6-OHDA injected into the cisternum magnum also produces a significant reduction (39% of control) in whole brain DA, $F(1,28)=90.837$, $p<0.001$.

H. Analysis of Heart NA

The means and SEs for the heart NA are shown in Table 1. A 2×2 ANOVA showed that only peripheral depletion

TABLE 1

MEANS AND STANDARD ERRORS OF WHOLE BRAIN DA, NA, WHOLE HEART NA (NG/G TISSUE) AND PLASMA 11-OHCS (μG/100 ML) FOR THE FOUR TREATMENT GROUPS

Treatment Groups	Whole Brain NA Mean ± SE	Whole Brain DA Mean ± SE	Plasma 11-OHCS Mean ± SE	Heart NA Mean ± SE
C_SP_S	396.7 ± 37.6	690.9 ± 50.5	43.8 ± 1.9	610.1 ± 30.5
C_SP_D	388.4 ± 31.7	586.0 ± 55.9	42.1 ± 2.2	170.1 ± 26.7
C_DP_S	118.2 ± 9.9	259.3 ± 28.8	40.8 ± 1.8	607.8 ± 31.5
C_DP_D	139.8 ± 5.5	236.8 ± 18.6	41.6 ± 3.9	170.8 ± 23.6

TABLE 2

PROBABILITIES OF THE DRUG EFFECTS ON THE BEHVIORAL MEASURES

Source	Avoidance responses over 10 days	Response Latencies over 10 days	Escape Latency over 10 days	Escape trials before 1st Avoidance over 10 days
Central (C)	*	*	†	*
Peripheral (P)	NS	NS	NS	*
C × P	NS	NS	NS	NS
Trials (T)	*	*	NS	*
C × T	†	*	NS	NS
P × T	NS	NS	NS	NS
C × P × T	NS	NS	NS	NS

*=$p<0.001$ NS=Non significant
†=$p<0.01$

effect was significant, $F(1,28)=254.9$, $p<0.001$, indicating that IP injection of 50 mg/kg of 6-OHDA produces significant reduction of peripheral NA (28% of control) at Day 10 levels.

Summary of Results

The pattern of results for all behavioral measures in the present experiment is summarized in Table 2. It can be seen from Table 2 that the main effect for central CA depletion for all the behavioral measures tested was highly significant indicating that overall performance, summed across trials or across days for all behavioral measures, in rats depleted of central CAs is significantly poorer than that of the control animals. Also, the significant interaction effects, Central CA depletion×Days, in avoidance responses and in response latencies indicates that the rate of acquisition for central CA depleted rats is significantly slower than that of the relevant control groups. Table 2 also shows that no significant main effect for peripheral NA depletion occurred in any of the behavioral measures tested except escape trials before the first avoidance response. The non-significant results indicate that the performance of peripheral NA depleted animals could not be differentiated statistically from the relevant control group performances.

DISCUSSION

Di Giusto and King [3] reported that animals depleted of peripheral NA by 6-OHDA do significantly worse in acquisition of a two-way shuttlebox avoidance task than saline-injected control groups; the earlier results of Oei and Associates [12,13] confirm this finding of Di Giusto and King [3]. However, the findings of both Di Giusto and King [3], Lord *et al.* [10] and Oei and King [12] are based on 70 acquisition trials in one learning session. As mentioned elsehwere [13] the significant retardation observed in those findings [3, 12, 13] could be a function of insufficient training trials. The present experiment using 10 daily sessions of 35 acquisition trials showed that across days animals depleted of peripheral NA increased their escape and avoidance performances significantly to the relevant control levels (Table 2). This suggests that rats depleted of peripheral NA eventually perform as well as the controls. Figures 1 and 2 clearly illustrate this outcome, e.g., on Day 8, peripheral NA rats (C_SP_D group) performed at almost the same level as the control animals (C_SP_S groups).

Figures 1 and 2 also indicate clearly that the performance of rats depleted of peripheral NA approximates that of the control group on Day 1. This is at variance with the results of Oei and King ([12], Experiment 2) showed that rats depleted

of peripheral NA performed significantly worse than did their controls. It must be pointed out that the Day 1 result of the present experiment was based on 35 acquisition trials and the result of Oei and King [12] was obtained from 70 acquisition trials. When the Day 1 result of the present experiment is compared to the first 35 acquisition trials reported by Oei and King [12] no significant differences emerged. However, when the data from Days 1 and 2 were analyzed in the same way as our previous studies [12,13], i.e., C_SP_S vs. C_SP_D and Trials 1 to 70 in blocks of 10, the differences are replicated showing that P_D animals performed more poorly than the control at Day 2. This suggests that the present Days 1 and 2 results are consistent with the results of our previous findings [12,13].

The present finding that rats depleted of peripheral NA required more escape trials before making the first avoidance response is not consistent with the results of previous findings [3,12]. This discrepancy could be due to the high mean number of escape trials before the first avoidance response in the C_DP_D group, particularly on Days 6–10 (Fig. 3). From Fig. 3 it can also be seen that the mean number of escape trials before the first avoidance response of the C_SP_D group almost dropped to the level of the control (C_SP_S) group on Day 6 and remained about that level to the end of the experiment. This suggests that the peripheral NA depleted animals do not need more escape trials before making the avoidance response.

The conclusion that is indicated by the present findings on peripheral NA depletion is that extended training ameliorates the observed behavioral deficit significantly back to the level of controls.

The biochemical evidence concerning heart NA concentrations indicates that peripheral NA was still significantly depleted (28% of control) even after 10 training days.

Even though the peripheral NA level was significantly depleted however, there was no observed behavioral deficit in the present experiment. This suggests that, in general, peripheral NA does not play a significant role in the recovery of function observed in the present experiment. Similarly, the biochemical evidence on plasma 11–OHCS shows that there was no significant difference between plasma 11–OHCS levels of control and peripheral NA depleted animals, which suggests that plasma 11–OHCS was not a mediating factor, at least not at the completion of training.

The significant findings for Central CA depletion on all the behavioural measures tested suggest that over 10 days training, the escape and avoidance performance of animals depleted of central CAs is significantly poorer than the control animals. These findings thus suggest that extended training does not significantly ameliorate the behavioral deficit in observed centrally CA depleted animals and are consistent with the findings of Cooper *et al.* [1] and Lenard and Beer [8,9].

The conclusions that are indicated by the present findings on central CA depletion are: (i) central CA depletion has a long term effect on the behavioral deficit. (ii) Comparison of the patterns of the significant behavioral differences between central CA and peripheral NA depletion (Table 2) leads to the further conclusion that central CA depletion is more detrimental to avoidance learning than peripheral NA depletion, given that percent depletion means the same functionally in the central and peripheral nervous systems.

As the previous findings [12,13] the statistical analysis of plasma 11–OHCS shows that there is no significant relationship between central CA depletion and plasma 11–OHCS levels after extended shuttlebox training. The present results thus failed to demonstrate that the observed behavioral deficits following central CA depletion by 6–OHDA were mediated by way of a central CA influence on plasma 11–OHCS. It thus seems reasonable to conclude tentatively that plasma 11–OHCS played no significant mediating role in the behavioral deficits observed in the present experiments.

Table 1 reveals a substantial decrement in the whole brain tissue level of NA (33% of control) and DA (39% of control). The results of statistical analysis indicate that these decrements in central NA and DA are each highly significant. Thus the biochemical evidence suggests that the depressed escape and avoidance performance in two-way shuttlebox avoidance after 10 days of training is related to decrements in the central CAs.

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