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p-Chloroamphetamine: Behavioral Effects of Reduced Cerebral Serotonin in Rats[1]

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VORHEES, C. V., G. J. SCHAEFER AND R. J. BARRETT. *p-Chloroamphetamine: behavioral effects of reduced cerebral serotonin in rats.* PHARMAC. BIOCHEM. BEHAV. 3(2) 279–284, 1975. – p-Chloroamphetamine (PCA), a serotonin depletor, was given to rats at least 24 h prior to testing in an open field or a shock avoidance Y-maze task. In the open field PCA groups showed hypoactivity and increased defecation up to 30 days after drug administration. These same animals plus independent groups of PCA animals, showed facilitated avoidance acquisition in the Y-maze up to 15 days after PCA administration. At the beginning of behavioral testing serotonin levels in PCA animals were reduced 70 percent and were still reduced 41 percent after 38 days in whole brain. These results suggest a separation between shock and non-shock effects of brain serotonin depletion. The facilitated avoidance also provides support for the role of serotonin as an inhibitory neurotransmitter mediating a behavioral suppression system.

p-Chloroamphetamine Serotonin Avoidance learning Open field

IT has been shown that reducing brain serotonin (5HT) with p-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, increases Sidman avoidance responding in rats [23]. Increased Sidman avoidance responding has also been found following treatment with p-chloroamphetamine (PCA) or p-chloromethylamphetamine (PCMA), which also reduce brain 5HT [12]. PCPA has likewise been found to facilitate the acquisition of active avoidance responding in rats [16,24].

In addition to shock avoidance tasks, the effects of 5HT depletion on general locomotor activity have been examined. Tenen [24], using stabilimeter cages, and Volicer [25], using observer ratings, found that PCPA produced a decrease in activity in rats. In contrast, Fibiger and Campbell [6], using three different activity measures (stabilimeter, running wheel and photocell cages) found PCPA increased activity in rats. Increased short term stabilimeter activity has also been found in rats following treatment with PCA [21].

Thus, the present investigation was undertaken in order to compare the effects of 5HT depletion on acquisition of active avoidance and activity measured in a shock avoidance task with activity in a nonshock situation where all other parameters were held constant and to follow the time course of these behavioral effects. PCA was chosen in preference to PCPA, the more commonly used tool for depleting 5HT, for the following reasons: (1) PCA produces a long lasting decrease in the activity of tryptophan hydroxylase and a significant reduction of both 5HT levels and turnover for up to four months and this effect is specific to brain [13, 14, 15]. (2) In PCA treated animals norepinephrine (NE) metabolism has returned to normal within 24 hours following drug treatment [21,22]. Therefore, in order to minimize NE influences on behavior produced by PCPA, animals were tested 24 hr or longer after treatment. It should be noted that although the mechanism by which PCA produces the effects cited above is still undetermined, the effects themselves are well established [8, 9, 13, 14, 15]. Behaviorally, an open field was used to measure locomotor activity in which shock was not employed and a Y-maze discriminated avoidance apparatus was used to measure avoidance acquisition and simultaneously record activity, a task in which there were shock contingencies.

METHOD

Animals

Eighty male Sprague-Dawley rats (Holtzman Co., Madison, Wis.), 77 days of age when the drug was first administered were used. Weights ranged from 312–390 g at the beginning of the experiment. Animals were housed one per cage on ad lib food and water in quarters maintained on a 12 hr light–dark cycle.

Apparatus

The open field consisted of a 122 × 122 cm plywood

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floor with 30.5 cm high surrounding walls. The interior surfaces were painted gray and the floor was marked off into 16 equal 30.5 x 30.5 cm squares with black lines. Illumination was provided by a single 60 W red light directly above the field. White noise was used to mask extraneous auditory stimuli and the room was maintained at 22–25.0°C.

The Y-maze has been described elsewhere [4]. Briefly, the fully automated Y-mazes consisted of three 28 x 18 x 15 cm arms with an opaque lid covering each arm. The walls were painted black and the three arms were joined by a 18 cm equilateral triangular choice area. The grid floor consisted of 0.6 cm wide bars spaced 2 cm apart on center. At the end of each arm there was a 7 W bulb which served as a cue light. A white noise generator provided the background masking noise. Animals' responses were recorded automatically by touch sensitive relays and photocells in each arm. Foot shock (1.25 mA, 60 Hz AC) was delivered to the grid floors through a scrambler regulated by an autotransformer. A fixed resistance (270 KΩ) in series with the animal provided relatively constant current.

Procedure

Animals were assigned to 1 of 2 treatment conditions and on three consecutive days were administered an IP dose of either 5.0 mg/kg D,L-p-chloroamphetamine hydrochloride in distilled water (PCA group) or an equivalent volume of distilled water alone (Control group).

Behavioral procedures. Beginning 1, 10, 20 or 30 days following the last injection separate groups (n = 5) from both the PCA and Control conditions were tested in the open field for 3 min/day for 5 consecutive days. Beginning 1, 5, 10 or 15 days following the last injection separate groups (n = 5) from both treatment conditions were tested in the Y-mazes for 25 trials per day for 6 consecutive days. Animals tested in the open field beginning 1 or 10 days following treatment were those tested in the Y-maze beginning 5 or 15 days, respectively, thereafter, in order to directly relate the open field and Y-maze behavioral changes in the same animals.

On each day of testing in the open field, animals were placed individually in the center of the open field and allowed to explore during the 3 min period. The number of squares entered with all 4 feet and the number of fecal boluses were counted for each animal.

On each day of testing in the Y-maze, animals were placed individually into the lighted arm with no shock on and the room darkened. A trial consisted of switching the stimulus light in random order to one of the dark arms. Entry into the lighted arm within 10 sec successfully avoided shock. Failure to enter the lighted arm within the 10 sec period resulted in shock onset, after which escape responses were possible. Shock remained on in the previously safe arm and the incorrect arm as well as in the center triangular choice area until the animal entered the lighted safe arm. If, during the intertrial interval, the animals left the safe arm and broke the photobeam at the entrance of either of the dark arms, shock was initiated in the dark arms and in the center section and remained on until the animal returned to the safe arm. The following response measures were recorded during each Y-maze session: (1) Avoidances: entry into the safe arm at any time during the 10 sec CS–UCS interval. (2) Activity: a measure of locomotor activity within the lighted arm during the intertrial interval. (3) Response Latency: time elapsed between onset of a trial and the animal's entry into the lighted arm. (4) Correct discriminations: when the initial response (whether escape or avoidance) was an entry into the lighted safe arm. (5) Incorrect Avoidances: when the initial response was an entry into the incorrect dark arm during the 10 sec CS–UCS interval. (6) Incorrect Escapes: entry into the incorrect dark arm following shock onset, shock remaining on until the animal responded by entering the lighted safe arm. (7) Intertrial Crossings: number of times the animal left the lighted safe arm to enter a dark arm and was shocked during the intertrial interval.

Biochemical procedures. At either 1 or 38 days following the last injection, separate groups from both PCA and Control conditions were sacrificed, brains removed and rapidly frozen for subsequent assay for whole brain 5HT according to the procedure of Bogdanski *et al.* [2]. The 38 day groups represented selected animals that had been tested in both behavioral tasks at varying intervals after drug administration.

RESULTS

All significant results reported below were at $p<0.05$.

Open Field

A 2 (treatment) x 4 (test interval) x 5 (days) analysis of variance was computed on the two response measures recorded in the open field.

Activity. The effect of days was not significant, therefore, each point in Fig. 1 (left) represents the mean number of squares entered averaged across the five days of testing. As can be seen the PCA animals were less active than the Controls which is reflected by a significant main effect of treatment, $F(1,32) = 7.26$. None of the other terms were significant.

Defecation. As with activity, defecation did not change significantly across days, thus, Fig. 1 (right) represents the mean number of fecal boluses averaged across the 5 days of testing. The main effect of treatments was significant, reflecting increased defecation for the PCA animals, $F(1,32) = 5.85$. Although the defecation rate diminishes at longer test intervals as seen in Fig. 1 (right) this effect was not significant due to the relatively large error variance for defecation scores.

Y-Maze

A 2 (treatment) x 4 (test interval) x 6 (days) analysis of variance was computed on each of the response measures in the Y-maze. Test interval was not a significant factor on the various response measures recorded in the Y-maze, therefore, this factor was not represented in Fig. 2 or 3 below.

Avoidances. Fig. 2 shows the mean number of avoidances by each group across days. The PCA group made significantly more avoidances than the Control group as reflected by a significant main effect of treatments, $F(1,35) = 4.69$.

Activity. Figure 3 shows the mean activity during the intertrial interval for both treatment groups. As can be seen, the PCA group was significantly more active than the Controls, $F(1,35) = 4.16$, reflecting the attenuated shock induced suppression in the PCA animals compared to Controls.

Latency. The PCA group had shorter response latencies (significant main effect) than Controls, $F(1,35) = 6.48$,

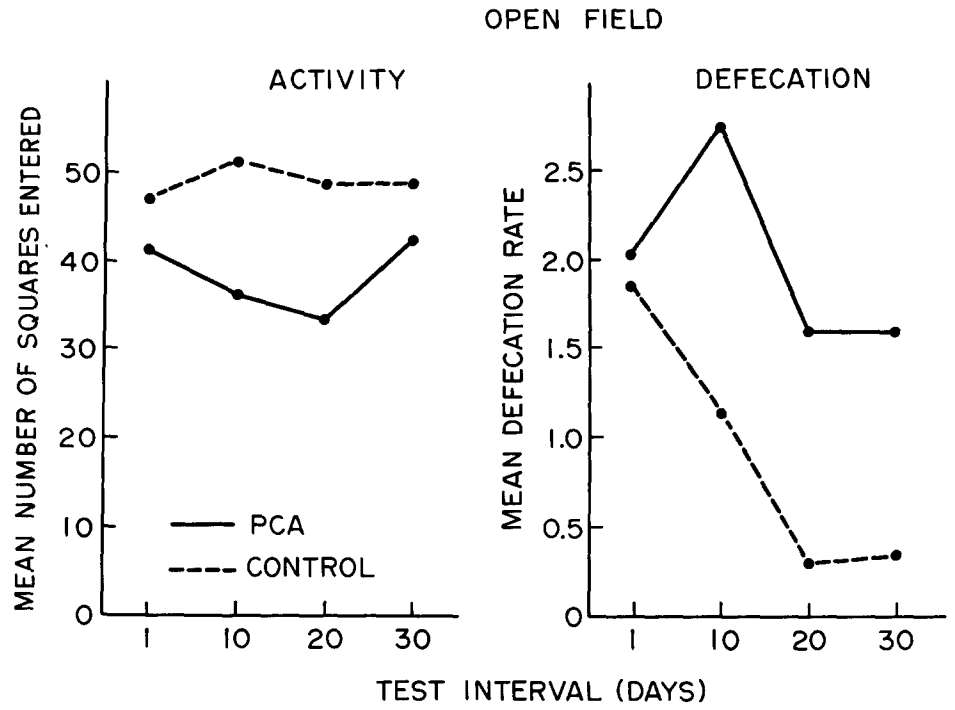

FIG. 1. Open field. Left, mean number of squares crossed averaged across animals and all 5 days of testing for each 3 min test interval. Right, mean number of fecal boluses averaged across animals and days for each 3 min test interval.

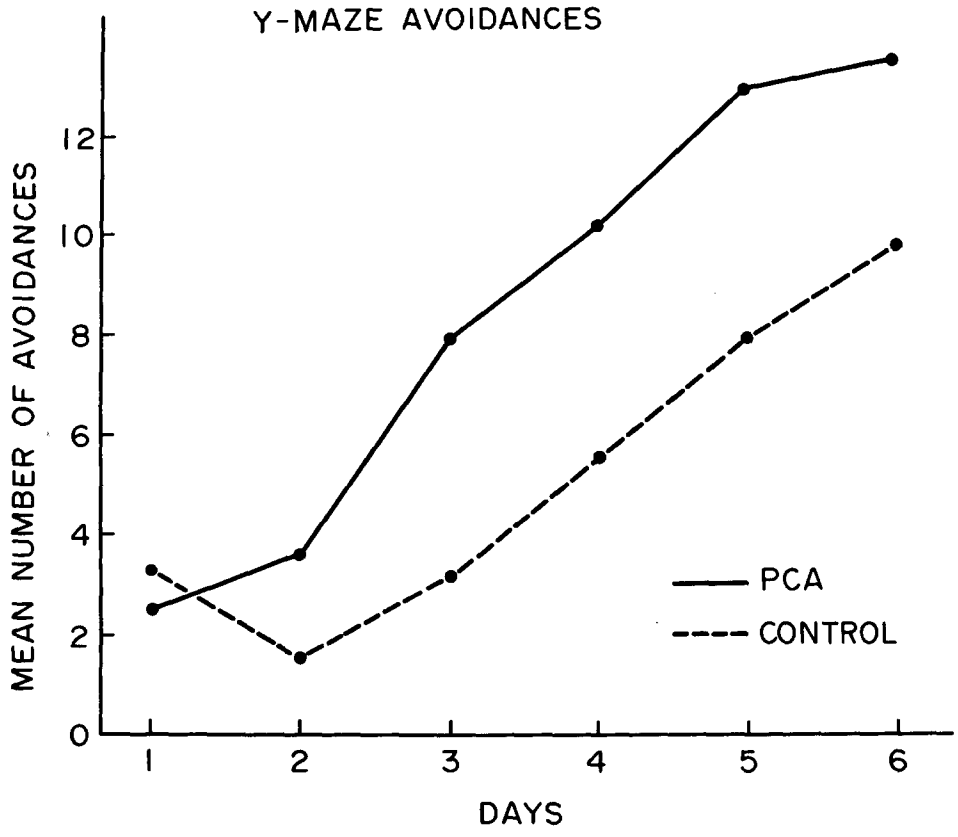

FIG. 2. Mean number of avoidances on each day of testing (25 trials/day) averaged across test intervals, i.e., animals tested beginning 1, 5, 10 or 15 days following drug treatment are pooled for each treatment (n = 20).

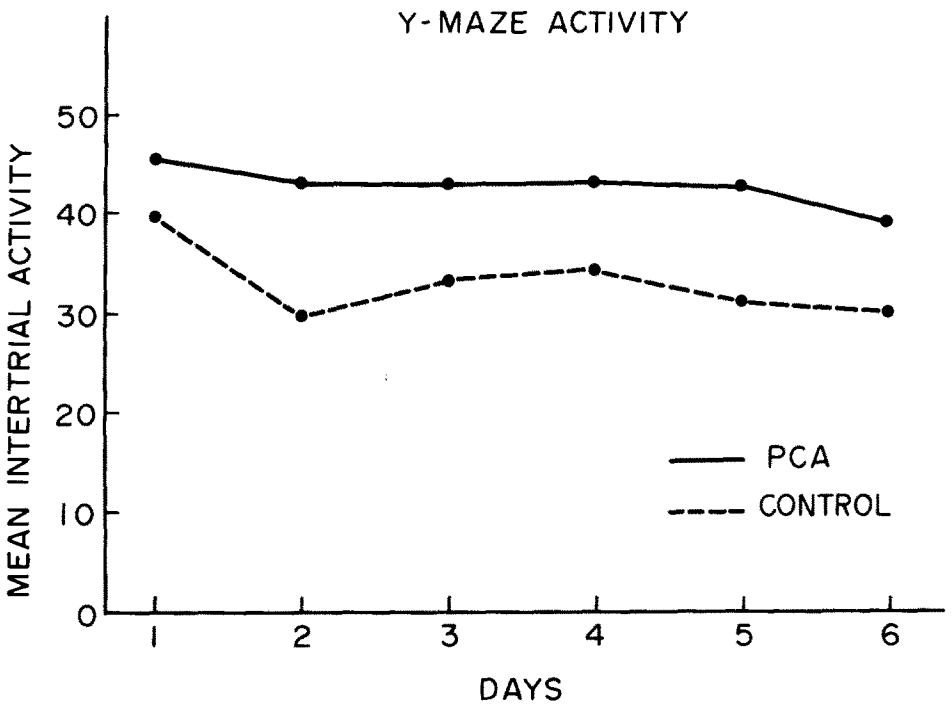

FIG. 3. Mean cumulative activity during the intertrial interval on each day of testing (25 trials/day). Animals tested beginning 1, 5, 10 or 15 days following drug treatment are pooled for each treatment (n = 20).

again reflecting the facilitated avoidance of the PCA animals.

Correct discriminations. There was no significant main effect of treatments on correct choices. There was, however, a significant Treatment x Days interaction, $F(5,175) = 3.23$, due to the PCA group initially making fewer correct discriminations, but by the sixth day of testing PCA animals were discriminating as well as Controls. Overall, correct discriminations indicated that both treatment groups learned to choose the safe arm equally well, reaching near perfect discrimination performance (93% correct) by the sixth day of testing.

Incorrect avoidances, incorrect escapes and intertrial crossings. These measures all reflect different types of errors. Incorrect avoidances reflect running at the right time but to the wrong place. Although the PCA group made more of these errors than Controls, this factor failed to reach a conventional level of statistical significance, $F(1,35) = 3.49$, $p = 0.067$. This trend is important because incorrect avoidances reflect the animals running tendency or activity, and therefore, is in line with the hyperactivity found in the PCA group during the intertrial interval. There were no significant treatment effects for either incorrect escapes or intertrial crossings.

5HT Levels

As can be seen in Table 1 5HT levels in PCA animals were reduced to 30% of Control levels at the beginning of behavioral testing ($t = 34.87$, $df = 18$) and after the end of the longest test interval at 38 days 5HT levels in PCA animals were still reduced to less than 60% of Controls ($t = 14.17$, $df = 10$).

DISCUSSION

The present results suggest that there is no simple relationship between 5HT depletion and general locomotor activity, whereas there is an inverse relationship between 5HT depletion and facilitation of active avoidance acquisition. Furthermore, the latter relationship is consistent with the proposed role of 5HT in mediating a behavioral suppression system [26]. Unfortunately, this framework is not consistent with the pattern of activity found in the open field and we are unable at present to fully resolve this inconsistency. However, the factors mediating motor activity in shock and non-shock tasks may differ and be dependent upon the baseline from which the experimental treatment changes the behavior and the specific response requirements of the task itself, factors that may be mediated by the balance between antagonistic serotonergic and adrenergic systems [3]. Accordingly, if reducing 5HT serves to disinhibit normal behavioral suppression, then the manifestation of this disinhibition should be most evident against a background of increased response suppression, as is produced by shock, whereas against a somewhat more neutral background, as in an open field situation, other factors may predominate and mask the disinhibiting effect of reduced 5HT. Thus, Ellison and Bresler [5] have found that PCPA treated rats were hypoactive in a novel quiet environment (an open field), but became hyperactive when stimulated by the introduction of lights and tones. Also Fibiger *et al.* [7] showed that the degree of stimulation induced hyperactivity produced by PCPA varied with the intensity of shock stimulation used.

Returning to the relationship between 5HT and performance in the Y-maze, it is clear that there is a consistent

TABLE 1

WHOLE BRAIN 5HT LEVELS

Days from Last Treatment to Assay*	Control	PCA	Percent of Control
1	0.64 ± 0.01 (9)†	0.19 ± 0.01 (11)	30
38	0.44 ± 0.01 (5)	0.26 ± 0.01 (7)	59

*PCA animals received 5.0 mg/kg PCA hydrochloride in three IP injections spaced 24 hr apart, while Controls received equivalent volumes of distilled water.

†Values represent group means expressed as μg/g ± S.E., with the number of animals per group in parentheses.

relationship between motor activity in the presence of shock and avoidance performance. It has been demonstrated [1] that avoidance acquisition depends upon some minimal level of initial activity in the shock situation. The activity level is important because it determines the probability, early in training, that the animal will make contact with the avoidance contingency. In rats in which shock normally elicits response suppression the likelihood of running prior to shock is small and avoidance acquisition is slow and incomplete. However, if 5HT mediates behavioral suppression, such as shock induced suppression, then diminished 5HT should disinhibit the animal, elevate baseline activity, and enhance avoidance acquisition. The higher incidence of incorrect avoidances and increased intertrial activity of PCA animals in the Y-maze provides independent evidence that these animals are more likely to run and, therefore, more likely to associate running with avoiding shock.

Further support for the above interpretation has been provided by other techniques, viz., depleting brain 5HT by electrolytic lesioning of the midbrain raphé nuclei, which contain most of the cell bodies of 5HT neurons [10]. Following recovery raphé lesioned animals show facilitated avoidance acquisition in the Y-maze compared to sham and non-treated controls and have substantially reduced forebrain 5HT levels [19]. These and other data based on raphé lesions [11] suggest that the avoidance acquisition seen in the present investigation is attributable to 5HT depletion rather than to some other effect of PCA.

The concept of a 5HT mediated shock induced suppression system is also supported by studies using passive avoidance. Depleting 5HT pharmacologically has been shown to disrupt passive avoidance avoidance [17, 20, 26].

Alternatively, the current results could be interpreted in terms of reduced 5HT producing an increase in emotionality or fear. This interpretation is consistent with the open field results since PCA treated animals were less active but defecated more than controls. In the Y-maze this interpretation implies that increased emotionality leads to increased reactivity to shock and hence facilitated avoidance in the PCA group. Unfortunately, however, in other contexts decreased emotionality or fear has also been used to explain facilitated avoidance acquisition. The fact that both increased and decreased emotionality have been used to account for avoidance facilitation, renders this notion somewhat meaningless as an explanatory concept [18].

Pharmacological evidence on the long-term effects of PCA suggest that a significant depletion of 5HT and reduction of brain tryptophan hydroxylase activity may persist for up to four months in adult rats after having received only a single dose of PCA [13]. Although no animals in the present study were tested as long as four months after treatment, preliminary data were obtained in the open field at intervals of up to one month. One of the striking features of the open field results was the consistency of the hypoactivity and increased defecation seen in the PCA treated animals even at the longest test interval.

Finally, because time since treatment (test interval) did not significantly alter the pattern of avoidance facilitation, it appears that there may be a critical level of 5HT reduction which produces optimal avoidance facilitation and beyond which there is little or no additional facilitation.

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