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BRIEF REPORT

p-Chlorophenylalanine-Produced Effects on Behavior In Intact and Brain-Damaged Rats[1]

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This study was undertaken to determine if a reduction in serotonin (5-HT) synthesis would provide any protection from the behavioral effects of hippocampal damage. The behavior of rats with such damage, of rats with only neocortical damage, and of sham operates was examined in two experimental situations. Half of each group had been treated with 300 mg/kg of *p*-chlorophenylalanine (PCPA) for 3 successive days before surgery, and half were given control injections of saline during this period. In the first group, locomotor, rearing, and grooming were observed over a 2-week period postoperatively in an open field. In the second group, rats were tested on the acquisition of a passive avoidance task 2 weeks after surgery. Animals with hippocampal damage became hyperactive in the second week of testing, but no reduction in this activity was produced by the drug administration. Rearing was initially depressed in animals with hippocampal damage, and PCPA facilitated its recovery but decreased the rearing of intact animals during the second testing week. Grooming levels were depressed throughout testing in animals with hippocampal damage. The PCPA treatment reduced grooming in animals with neocortical damage. Acquisition of the passive avoidance was impaired for groups with hippocampal damage.

There have been several demonstrations that the biochemical intervention before or just after damage to the central nervous system can reduce the usual consequences of this damage (Berger *et al.*, 1971, 1973; de Castro and Balagura, 1976; Glick and Greenstein, 1974; Glick *et al.*, 1972;

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Harrell and Balagura, 1975; Hynes *et al.*, 1975; Lanier *et al.*, 1974). The interpretations of these changes in the behavioral consequences of brain damage are varied but include the reduction of norepinephrine released near the site of the damage (Osterholm and Mathews, 1972a,b) and the induction of a chemical supersensitivity in certain remaining systems prior to the induction of additional supersensitivity produced by the lesion itself (Glick and Greenstein, 1974). The present study was undertaken to determine if a reduction in serotonin (5-HT) activity would provide any relief from the usual effects of hippocampal damage using an experimental procedure similar to that used by Lanier *et al.* (1974). In that study animals pretreated with AMT prior to bilateral hippocampal destruction had a less extreme passive avoidance deficit than those not given AMT, although the locomotor hyperactivity that develops about 1 week postoperatively was not affected by the AMT pretreatment. Changes in the usual consequences of hippocampal damage after prior serotonin depletion would also be of interest because of the possibility that the presence of 5-HT in catecholaminergic neurons may be responsible for their capability for sprouting (Berry and Riches, 1974).

The adminstration of *p*-chlorophenylalanine (PCPA) depletes 5-HT content of the brain by interfering with the enzyme, tryptamine hydroxylase (Koe and Weissman, 1966; Weissman, 1973). This prevents the synthesis of serotonin. In the present study, rats were treated with PCPA for 3 days prior to receiving bilateral hippocampal or neocortical lesions. Testing for changes in locomotor activity in the open field started immediately after surgery. Ten open field tests were conducted over the subsequent 2 weeks. During the testing in the open field, grooming and rearing behavior were also recorded. After the testing in the open field, the rats were trained on a passive avoidance task.

On the basis of three 5-min preoperative tests performed on consecutive days in the open field, 43 male Long–Evans hooded rats from Charles River Farms were divided into six groups with approximately equivalent mean activity scores. Intraperitoneal injections of an aqueous suspension of PCPA (100 mg/kg at 10 mg/ml each day, total of 300 mg/kg) were given to nine rats that were to receive bilateral hippocampal ablations (Group Hp), six rats that were to receive bilateral neocortical lesions (group Np) and to eight intact, normal rats (Group Cp). Saline (pH corrected) injections were given to an additional eight rats that were to receive hippocampal ablation (Group Hc), to four rats that were to receive neocortical lesions (Group Nc), and to seven intact rats (Group Cc). These injections were given in the morning of 3 consecutive days immediately following the preoperative activity test and before the day of surgery. These dose levels of PCPA have been shown to block the synthesis of 5-HT (Koe and Weissman, 1966) and reduce the levels of serotonin by over 80% of controls.

All rats, including unoperated controls, were given 50 mg/kg of sodium

pentobarital anesthesia. The hippocampal lesions were made by aspiration using the techniques described by Isaacson and Woodruff (1975). These procedures resulted in bilateral lesions, which involve removing 60–90% of the hippocampus as well as removing part of the overlying neocortex. At the end of the experiment the rats were perfused with saline and 10% formalin solution. The brains were embedded in celloidin, and 20-μm sections were cut and stained in thionin. The size and location of the lesions were found to be comparable with those produced in many studies originating in this laboratory (e.g., Woodruff and Isaacson, 1972).

Five-minute open field activity tests were given from the day after surgery through the fifth day and on every other day thereafter for a total of 10 postoperative sessions. The open field measured 1 × 1 m and it was subdivided into 49 squares of equal area. The measure of activity was the number of squares crossed in the 57-min test period. The open field arena was illuminated by one 40-W lamp 80 cm above the floor of the arena. The rest of the room was dark. Rearing, grooming, and defecation were also recorded by an observer on a multichannel recorder in the darkened room.

Beginning on the 14th day after surgery, all animals were placed on a 23-hr water deprivation schedule. On the fourth day of deprivation each rat was given a 10-min adaptation period in the passive avoidance apparatus. This was a wooden box (60 × 21 × 31 cm) painted gray with a wooden door through the middle, which could be raised to allow movement between the two halves of the box. The floor was a metal grid. During the adaptation period, the door was raised and water was available at one end.

On the following day (the first day of training) 10 trials were given. Three minutes were allowed for each trial. A maximum time of 10 sec of drinking was permitted on each trial. The latencies to enter the second compartment to drink were recorded. The intertrial interval between removal from the drinking compartment, replacement in the starting compartment, and raising of the door between compartments was about 15 sec. One the second day of training, five more trials were given. On trials 6–10 the rat received an electric footshock of 0.5 mA on entering the second compartment.

All between-group comparisons were made by Mann–Whitney U tests, and probabilities are for two-tailed tests on independent samples. All intragroup probabilities were determined according to the Wilcoxon matched-pairs test.

Both groups of animals with hippocampal damage became hyperactive in the open field by the second week after surgery compared to their preoperative levels of activity ($P < 0.01$, Fig. 1). Activity levels were higher for animals with hippocampal lesions than for those with neocortical damage ($P < 0.05$) and for unoperated controls ($P < 0.002$) in this second week. Rearing was depressed in animals with hippocampal dam-

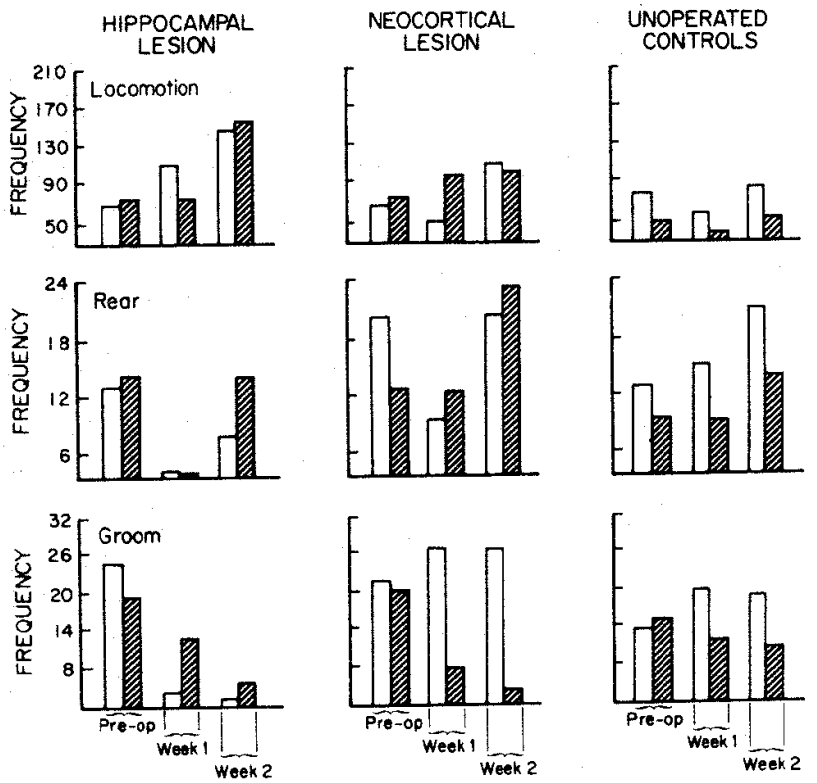
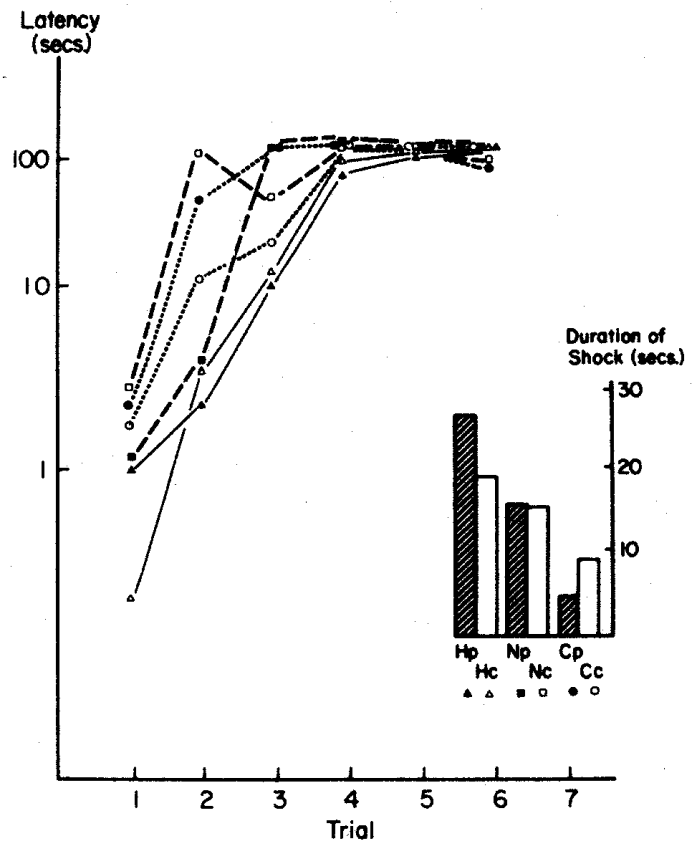


FIG. 1. The mean preoperative and postoperative frequencies of locomotion, rearing (Rear), and grooming (Groom) for groups of animals with hippocampal lesions and neocortical lesions and for operated controls. The shaded bars represent animals treated with PCPA before surgery. Open bars are those treated with saline.

age in the first week after surgery ($0.02 > P > 0.002$), but partially recovered during the second postoperative week. The animals with hippocampal damage still reared less than those with neocortical damage in the second week following surgery ($0.05 > P > 0.025$). Animals with hippocampal lesions pretreated with saline reared less than they had preoperatively ($P < 0.02$), but those pretreated with PCPA returned to preoperative levels. During the second week the PCPA-treated intact control animals reared less than similar animals that received saline treatment ($P < 0.05$). All animals with hippocampal damage, as well as animals with only neocortical damage and PCPA treatment, showed reduced amounts of grooming compared to their preoperative levels, the grooming of controls ($P < 0.05$), and their saline-treated counterparts ($P < 0.038$).

Both groups of animals with hippocampal damage had deficits in the passive avoidance task. They continued to approach the water on more trials than did other groups of animals and entered the shock compartment with shorter latencies than intact animals by the second shock trial ($0.02 > P > 0.002$) and with shorter latencies than animals with neocortical damage by the third shock trial ($P < 0.02$) (see Fig. 2).



The mean postoperative response latencies of all groups in the passive avoidance task. Inset shows mean duration of shocks received by each group. Hippocampal lesions treated with PCPA (Hp), closed triangles, shaded bars; hippocampal lesions treated with saline (Hc), open triangles, open bars; neocortical lesions treated with PCPA (Np), closed squares, shaded bars; neocortical lesions treated with saline (Nc), open squares, open bars; intact controls treated with PCPA (Cp), closed circles, shaded bars; intact controls treated with saline (Cc), open circles, open bars.

Since the shock was delivered for as long as animals remained in the compartment with the water, it is possible to compare the total amounts of shock each group received over the six trials. The groups with hippocampal damage received more shocks than those with neocortical damage ($P < 0.05$) and intact groups ($P < 0.002$). Over the six shock trials of Day 1, PCPA-treated intact animals received less shock than those treated with saline ($P < 0.036$). The animals with neocortical lesions were not affected by the PCPA treatment. The PCPA treatment increased the amount of shock the animals with hippocampal lesions recieved when evaluated against the performance of intact animals by the Moses test of extreme reactions (Siegel, 1956). These results support the latency data in showing that animals with hippocampal damage are impaired in withholding approach responses in a passive avoidance task.

The treatment of animals with PCPA failed to protect animals with bilateral hippocampal damage from developing the alterations in behavior

usually found after such damage. The animals with hippocampal lesions developed hyperactivity at about the anticipated postoperative time (second week after surgery) and were impaired in acquisition of a passive avoidance response. It was in the passive avoidance task that some decreased impairment was found after pretreatment with the catecholamine depletor AMT by Lanier *et al.* (1974).

The effect of PCPA treatment on brain 5-HT levels was not measured in the present experiment due to the extended testing of all subjects, but the data reported by Koe and Weissman (1966) indicate that after similar treatment 5-HT returns to control levels about 2 weeks after being reduced to less than 20% of control values in the first postinjection week and about 60% of control values in the second postoperative week.

The failure to find PCPA effects on the locomotor behavior of intact animals is consistent with the lack of locomotor changes found by Isaacson *et al.* (1977) for adult rats with about 70% reduction in forebrain 5-HT levels after intracisternal 5,6-DHT administration at 5 days of age. Locomotion in an open field depends on the activity in specific ascending 5-HT tracts (Srebro and Lorens, 1975) rather than the brain content of 5-HT remaining after the lesion. These authors found that lesions of the medial raphé nuclei produce decreases in locomotor activity with only a 26% decrease in forebrain 5-HT. Damage to other raphé nuclei failed to alter locomotor behavior despite much greater reductions in forebrain 5-HT content.

A reduction in grooming was not found after hippocampal lesions by Kim *et al.* (1970) or Jarrard (1968) in rats or by Glickman *et al.* (1970) in the gerbil. In all of these studies, however, grooming was observed in the home environments of the animals. In this study grooming was evaluated in a relatively novel, open field arena. It is possible that the act of being transported to the arena or its novelty acts to induce grooming in the control animals but not those with hippocampal lesions.

The hyper-responsiveness to footshock that has been reported as a consequence of PCPA (e.g., Harvey and Yunger, 1973) and some residual effect of PCPA may account for the fact that the control group treated with PCPA received fewer shocks than the nontreated control group during acquisition of the passive avoidance task. However, this was not found in animals with either hippocampal or neocortical damage. This supports the suggestion that certain serotonergic influences are mediated by dorsal hippocampal areas (Jacobs *et al.*, 1975).

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