Pharmacology Biochemistry and Behavior, Vol. 1, pp. 609–613. ANKHO International Inc., 1973. Printed in the U.S.A.

The Effects of Parachlorophenylalanine on Experimentally Induced Conflict Behavior[1]

THOMAS A. BLAKELY AND LORNE F. PARKER[1]

Department of Psychology, University of Washington, Seattle, Washington 98195

(Received 1 March 1973)

BLAKELY, T. A. AND L. F. PARKER. *The effects of parachlorophenylalanine on experimentally induced conflict behavior.* PHARMAC. BIOCHEM. BEHAV. 1(6) 609–613, 1973. Rats were subjected to an experimentally induced conflict procedure in which a metronome-signaled punishment period was superimposed upon a fixed ratio schedule of bar pressing for food; the punishment consisting of electric shock to the feet. Administration of parachlorophenylalanine three days before testing was found to have no effect upon the number of punishments accepted. This finding was interpreted as suggesting that, due to the large within-animal variability observed by these and other experimenters, the reported efficacy of parachlorophenylalanine in enhancing acceptance of punishment may be questionable.

Para-chlorophenylalanine 5-Hydroxytryptophan Punishment Conflict

THE DISCOVERY of Koe and Weissman [7] that parachlorophenylalanine (pCPA) produces a selective depletion of brain serotonin has led to the widespread use of this amino acid in the study of the role of serotonin in the central nervous system. In a series of experiments by Tenen [9] serotonin depletion by pCPA was shown in increase sensitivity to painful shock in rats and to facilitate avoidance learning in an active avoidance situation. This pCPA-induced facilitation of avoidance learning was shown to be due to increased shock sensitivity.

Recently, Robichaud and Sledge [8] tested the effects of pCPA on rats in the experimentally induced conflict paradigm of Geller and Seifter [5]. These investigators found that pCPA injections enhanced response perseveration when food reinforced bar pressing was simultaneously punished with shock. In a similar experiment, Geller and Blum [4] likewise found the pCPA injections enhanced shock acceptance and, in addition, this effect of pCPA was reversed by administration of 5-hydroxytryptamine (5-HTP), the biological precursor of serotonin.

Brody [2,3], however, failed to find any significant enhancement of shock acceptance after pCPA injections unless the drug was administered both three days and one hour before testing. Since serotonin was effectively depleted at the time of the second injection, the effect of pCPA on shock tolerance may have been independent of its serotonin-depleting activity.

These contradictory findings, along with the reported increases in shock sensitivity produced by serotonin depletion [9], make it questionable that serotonin depletion via pCPA injections should enhance shock acceptance. Furthermore, the great within-group variance reported by Robichaud and Sledge [8], and Geller and Blum [5], coupled with their lack of statistical analysis, suggests that the pCPA induced enhancement of shock acceptance they reported may have not been statistically reliable.

Thus, the present study was designed to determine the effects of pCPA and 5-HTP on shock acceptance in an experimentally induced conflict situation. The procedure employed differs from that of Geller and Seifter [5] in two important ways: (1) a very high rate of responding was produced in all animals by using a fixed ratio schedule of reinforcement; (2) the experiment was designed to allow the use of inferential statistics. The high rate of responding was used to accentuate suppression and enhance possible differences in suppression between groups. The employment of statistical analysis was important in that statistical measures of reliability were not used by any of the authors cited above except Brody [2,3]; the only one to report negative findings.

METHOD

Animals

Twenty-six male Long-Evans rats were used. The animals were obtained from the colony maintained by the Department of Psychology of the University of Washington, and were between 90 and 120 days old at the initiation of the experiment. All animals were housed individuallly in stainless steel cages and had access to water throughout the experiment.

[1] The authors thank Curtis Harris for valuable technical assistance. Reprint requests should be directed to Lorne Parker, Department of Psychology, University of Washington, Seattle, Washington, 98105, U.S.A.

Drugs

The drugs used in this experiment (the pCPA was generously supplied by Dr. Albert Weissman of Chas. Pfizer and Company) were suspended in a five per cent solution of gum acacia and administered via intraperitoneal injections (IP). The dose of pCPA was 340 mg/kg and the dose of 5-HTP was 75 mg/kg. Where control injections of vehicle were made, the volume was equivalent to that of the drug injections.

Behavioral Assessment

Eighteen rats, randomly assigned to one of three groups (n = 6 per group), were used to determine the behavioral effects of the drugs. The animals were reduced to 75 to 80 percent of their ad lib body weights by restricted feedings and were then trained to bar press for food pellets on a FR-12 schedule of reinforcement. Training was considered completed when the animal pressed consistently at a rate of at least 20 responses per min. Following completion of bar press training the animals were given daily 19 min bar press sessions on the FR-12 schedule for six days. At the end of the eighth minute of each session a metronome (30 pulses per sec at 65 db) was turned on for three min. On Days 1, 2, and 3 of these sessions the animals were given habituation trials with the metronome and no shock. On Days 4 and 5 all the animals had 0.5 mA of shock for all bar presses made during the metronome, accompanying food reinforcement on the same FR-12 schedule. On Day 6 the shock level was raised to 0.6 mA and, since this level produced a moderate to high degree of response suppression across animals, it was maintained for the rest of the experiment. On Day 7 the animals were injected with either pCPA or vehicle, depending upon their treatment group, and tested in the bar press apparatus one day, three days, and nine days after the injections. The animals were also injected with either 5-HTP or an equivalent volume of vehicle one hour prior to being tested.

Group 1 animals received pCPA in the initial injection, and vehicle in all three pretrial injections. Group 2 animals received pCPA in the initial injection, vehicle in the first pretrial injection (one day after pCPA), 5-HTP in the second pretrial injection (three days after pCPA), and vehicle in the last pretrial injection (nine days after pCPA). Group 3 animals received vehicle in all injections.

The rationale for these groups lies in the fact that if there was an effect due to serotonin depletion by pCPA, this effect would be reversed by injection of 5-HTP at the time of peak effect of the pCPA injection, i.e., three days after pCPA injection. Thus, Group 1 animals were serotonin depleted during all three test days, Group 2 animals were serotonin depleted only on the first test day, and Group 3 animals were not serotonin depleted during any of the test days.

Bar presses, reinforcements, and presentation of the auditory stimulus were recorded on a Gerbrands cumulative recorder. (Fig. 1) The data consisted of suppression ratios computed by the formula:

$$SR = \frac{A - B}{A}$$

Where:

SR = suppression ratio
A = presses three min before the punishment period
B = presses during the punishment period

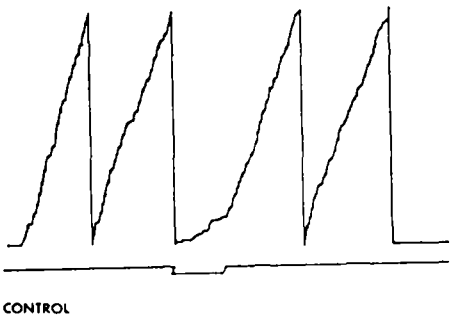

FIG. 1. Representative cumulative record of bar pressing during FR-12 schedule of reinforcement and during punishment period (depression in abscissa).

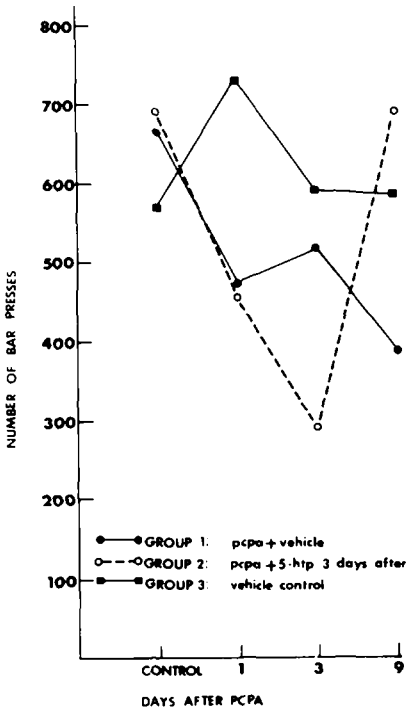

FIG. 2. Total number of unpunished bar presses during each session. Each value represents the mean for six animals.

Note that a ratio of · 1 indicates total suppression and a ratio of 0 indicates no suppression.

Biochemical Assessment

In order to verify that injections of pCPA had the expected biochemical effects, an additional eight rats were randomly divided into four groups of two rats each. The rats received two injections: one three days, and a second one hour before they were sacrificed by rapidly cutting the carotid vessels. The four drug treatments for the three day and one hour injections respectively were: (1) pCPA and vehicle; (2) pCPA and 5-HTP; (3) vehicle and 5-HTP; and, (4) vehicle and vehicle. Although the third treatment, vehicle and 5-HTP, had no direct relevance to the behavioral experiment, it was included as an added check on the efficacy of the 5-HTP. The brains were frozen in a dry

TABLE 1

ANALYSIS OF VARIANCE FOR REPEATED MEASURES FOR RATES OF LEVER PRESSING

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
Between Animals	3,598,834	17		
A (Groups)	191,432	2	95,716	0.421
Subjects within groups	3,407,402	15	227,161	
Within Animals	2,049,845	54		
B (Days)	277,911	3	92,637	4.164*
AB (Groups X days)	770,785	6	128,464	5.774†
B X subjects within groups	1,001,150	45	22,248	
Simple Main Effects				
A at B_1	45,512	2	22,756	0.310
A at B_2	317,960	2	158,980	2.164
A at B_3	310,281	2	155,140	2.111
A at B_4	288,464	2	144,232	1.963
Within Cells	4,408,552	60	73,476	
B at A_1	263,498	3	87,833	3.948*
B at A_2	675,369	3	225,123	10.119†
B at A_3	112,147	3	37,382	1.680
Days	3	4	2	1
Means	468.7	549.8	554.2	724.8
3	–	81.1	85.5	256.1†
4		–	4.4	175.0†
2			–	170.6†

*$p = 0.05$ †$p = 0.01$

ice-ethanol bath and stored at --25 degrees centigrade. They were assayed 24 hr later for serotonin content using the method of Bogdanski, Pletcher, Brodie and Udenfriend [1].

RESULTS

Behavioral Findings

Injection of pCPA appeared to make animals slightly more sensitive to being handled. Aside from this, neither animals receiving pCPA alone nor animals receiving 5-HTP in combination with pCPA showed any obvious differences from control animals. All animals appeared to eat normally when given free access to food following the daily sessions.

The mean number of total bar presses for each group on each experimental day are shown in Fig. 2. As depicted, both drug injected groups showed somewhat lower rates of lever pressing with the lowest rates being observed in the animals injected with 5-HTP one hour before testing. Overall between group differences in bar pressing rates were analyzed using analysis of variance for repeated measures, as described by Winer [10]. This analysis is shown in Table 1. The significant main effect of factor B (days) was further analyzed using the Neuman-Keuls procedure for the differences between all possible pairs of treatment means. The significant differences between Day 1 (the control day) and all other days indicates that the significant main effect of factor B was due to a significant tendency for a reduction in the number of lever presses on drug injection days. The significant simple main effects B at A_1 and B at A_2 indicate that the significant AB (groups x days) interaction was due to a significant reduction in lever pressing by the two drug injected groups, which was not shown by the vehicle injected group.

The mean suppression ratios for each experimental test day are shown in Fig. 3. Inspection of this figure reveals that the drug injections had no significant effect on the suppression ratios.

Biochemical Findings

Lack of a significant drug-induced effect on suppression

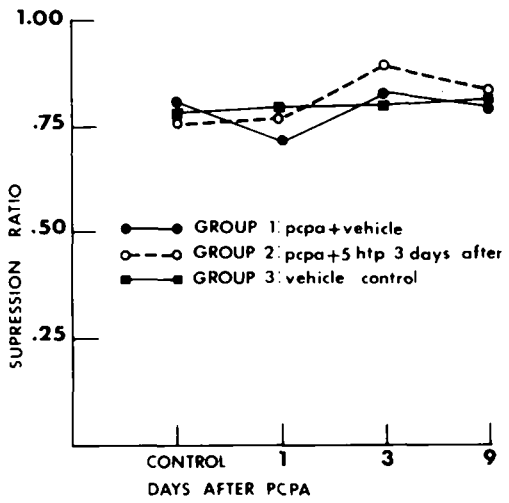

FIG. 3. Performance on discriminated punishment. Each point represents the mean for six animals.

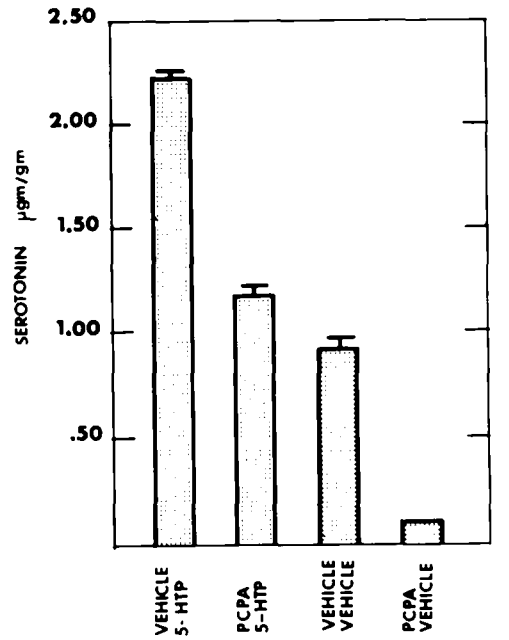

FIG. 4. Serotonin levels after injection of pCPA, 5-HTP, and both in combination. Each value is the mean for two animals. Brachets represent range of values.

ratios in this experiment could conceivably have been due to failure of the drugs to produce the expected biochemical effects as a result of improper techniques of storage, preparation, or injection. However, the results of the brain assays, shown in Fig. 4, indicate that this was not the case. Parachlorophenylalanine was found to produce marked depletion of brain serotonin and 5-HTP was found to produce marked elevation of brain serotonin. These data were analyzed using analysis of variance with the Neuman-Keuls procedure for comparing all possible pairs of treatment sums, as described by Winer [10]. As shown in Table 2, all differences were found to be significant at the 0.01 level except for the difference in serotonin content of the pCPA-5-HTP group and the vehicle-vehicle group i.e., injections of 5-HTP three days after injections of pCPA were found to bring brain serotonin levels back to normal.

The level of serotonin content in the control animals and the degree of depletion produced by pCPA are comparable to those reported by Koe and Weissman [7], and Jequier, Lovenberg, and Sjoerdsma [6]. Hence, the drug injections produced their expected effects on brain serotonin content.

DISCUSSION

In general, the findings of this experiment fail to support the contention of Robichaud and Sledge [8], and Geller and Blum [4] that depletion of brain serotonin, via injections of pCPA, enhances the rat's willingness to accept shock in an experimentally induced conflict situation. That is, the animals in Group 1 of this experiment, which received pCPA three days before testing, showed only a very slight increase in bar press suppression which was not statistically significant.

There are several differences between their studies and the present one which might conceivably account for this discrepancy in findings. First, Robichaud and Sledge [8], and Geller and Blum [4] used a 2 min variable interval schedule of reinforcement, which was changed to continuous reinforcement during each of four punishment sessions. This procedure may have produced a higher motivational level during the punishment intervals than the fixed ratio schedule employed by the present authors. A second possibility is that the reward they used, sweetened condensed milk, may have had a different incentive value than the dry food pellets used in this experiment.

However, if depletion of brain serotonin via pCPA injections does in fact reduce the animal's inhibitions in a conflict situation, as Robichaud and Sledge [8] suggested, this phenomenon should be demonstrable using varying schedules and types of reinforcement. Furthermore, the large within-animal variability reported by Robichaud and Sledge [8], and Geller and Blum [4] make it likely that if their experiments had been designed to allow statistical analysis it would not have been possible to demonstrate a reduction by pCPA of shock-induced suppression in the experimentally induced conflict situation to a statistically significant degree.

One important difficulty with previous reports of the effects of drugs on shock-induced suppression of lever pressing is that the data have been presented as the number of punishments accepted during the experimental session, with no attempt being made to evaluate the effect of the shock contingency on the animal's on-going rate of lever pressing. Geller and Blum reported that pCPA injected rats were willing to tolerate fewer shocks after an injection of 5-HTP. These authors, however, failed to provide any information about the overall rates of lever pressing, and since they reported suppression as the number of lever presses made during the punishment intervals rather than as suppression ratios, there is no way of knowing whether the observation that 5-HTP injected rats accepted fewer punishments was really due to greater response suppression during the punishment intervals or if it was just a reflection of an overall decrease in the rate of lever pressing. The significant reduction in the rate of lever pressing following 5-HTP injection found in the present experiment suggests that the rate of lever pressing during the punishment interval should

TABLE 2

ANALYSIS OF VARIANCE FOR SEROTONIN LEVELS

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
Total	4.668	7		
Treatment	4.583	3	1.528	71.217
Error	0.0858	4	0.0215	
Order	1	2	3	4
Treatment	pCPA Vehicle	Vehicle Vehicle	pCPA 5-HTP	Vehicle 5-HTP
Sum	0.266	1.89	2.45	4.50
pCPA, Vehicle	–	1.624†	2.184†	4.234†
Vehicle, Vehicle		–	1.624†	2.610†
pCPA, 5-HTP			–	2.610†
Vehicle, 5-HTP				–

*$p = 0.05$ †$p = 0.01$

be evaluated in relationship to the rate during the rest of the experimental session. This can easly be done by reporting the data as suppression ratios and by analyzing the effects of the drugs on the total number of lever presses.

The present findings show that injections of pCPA and of 5-HTP in combination with pCPA, which had highly significant effects on brain serotonin levels, failed to have significant effects on the rat's willingness to accept punishment in an experimentally induced conflict situation. Although there were no significant effects on suppression ratios, the drug injections, particularly the injection of 5-HTP, were found to significantly reduce the total number of lever presses during an experimental session.

REFERENCES

1. Bogdanski, D., A. Pletcher, B. Brody and S. Udenfriend. Identification and assay of serotonin in the brain. *J. Pharmac. exp. Ther.* **117:** 82–88, 1956.
2. Brody, J. F. Jr. Behavioral effects of serotonin depletion and of p-chlorophenylalanine (a serotonin depleter) in rats. *Psychopharmacologia* **17:** 14–33, 1970.
3. Brody, J. F. Jr. Effects of p-chlorophenylalanine (a serotonin depleting agent) on behavior. Unpublished Ph. D. dissertation, University of Pittsburg, 1969.
4. Geller, L. and K. Blum. The effects of 5-HTP on parachlorophenylalanine (p-CPA) attenuation of "conflict" behavior. *Eur. J. Pharmac.* **9:** 319, 1970.
5. Geller, I. and J. Seifter. The effects of meprobamate, barbituarates, D-amphetamine, and promazine in and experimentally induced conflict in the rat. *Psychopharmacologia* **1:** 482, 1960.
6. Jequier, E., W. Lovenberg and A. Sjoerdsma. Tryptophan hydroxylase inhibition: the mechanism by which p-chlorophenylalanine depletes rat brain serotonin. *Molec. Pharmac.* **3:** 274–278, 1967.
7. Koe, B. and A. Weissman. P-Chlorophenylalanine, a specific depleter of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
8. Robichaud, R. and K. Sledge. The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. *Life Sci.* **8:** 965–969, 1969.
9. Tenen, S. The effects of parachlorophenylalanine, a serotonin depleter, on avoidance acquisition, pain sensitivity, and related behavior in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
10. Winer, B. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.