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5-Hydroxytryptamine Depletion with Para-Chlorophenylalanine: Effects on Eating, Drinking, Irritability, Muricide, and Copulation[1]

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PAXINOS, G., J. BURT, D. M. ATRENS AND D. M. JACKSON. *5-Hydroxytryptamine depletion with para-chlorophenylalanine: effects on eating, drinking, irritability, muricide, and copulation.* PHARMAC. BIOCHEM. BEHAV. 6(4) 439–447, 1977. – Forty-four male rats were tested for eating, drinking, irritability, and copulation before and after intraperitonial para-chlorophenylalanine (PCPA) or control injections. Eleven of these rats were tested for muricide before and after PCPA injections (Group 1), while 18 others were tested only after PCPA injections (Groups 2). Group 1 rats received four 350 mg/kg PCPA injections spaced 6 days apart and showed hyperdipsia, weight loss, and a 24% increase in muricide. Group 2 rats received five daily 100 mg/kg PCPA injections repeated 11 days later and showed hyperdipsia and weight loss; in addition, 78% of them killed mice. Neither group showed significant changes in copulation. At the end of the experiment, 6 rats from Group 2 that were irritable and killed mice were injected intraperitonially with 5-hydroxytryptophan (80 mg/kg). Five of these rats lost their irritability and four stopped killing. The various behavioral changes were not correlated significantly either with each other or with the degree of 5-hydroxytryptamine depletion. This tentatively suggests that PCPA may produce its effects on behavior by other means in addition to 5-hydroxytryptamine depletion.

Eating Drinking Irritability Muricide Copulation 5-Hydroxytryptamine
Para-chlorophenylalanine 5-Hydroxtryptophan

IN RATS, para-chlorophenylalanine (PCPA) injections deplete brain 5-hydroxytryptamine (5-HT) levels by inhibiting tryptophan hydroxylase, the rate-limiting enzyme in the formation of 5-HT from dietary tryptophan [16,19]. PCPA injections have also been shown to deplete brain norepinephrine (NE) and dopamine (DA) levels, but the depletion of the catecholamines (CA) is smaller and of a shorter duration than that of 5-HT. Thus, Koe and Weissman [19] found that 3 days after a single 316 mg/kg dose of PCPA the 5-HT depletion in the brain reached a maximum of 93%. 5-HT concentrations remained more than 50% depleted for 8 days after the injection and did not return to control levels for about 2 wk. Koe and Weissman found that the same PCPA dose reduced NE by about 20% during the 6 days after injection. Miller *et al.* [26] gave a 400 mg/kg injections of PCPA and obtained a maximum 5-HT depletion of 68% and a maximum NA depletion of 30%. In their study, 5-HT and NA were significantly lowered for the first 12 and 5 days, respectively. Like NE, DA is also depleted by about 20% for the first few days after PCPA injections, but it returns to control levels within 5 days [13,19].

PCPA has often been used to investigate the involvement of 5-HT in the control of behavior because of its somewhat specific 5-HT depleting effect. Many consistent behavioral changes have been reported to result from PCPA injections; however a number of inconsistent findings have also been reported. A small decrease in food intake and a resulting weight loss are typically reported following PCPA injections [6, 25, 43], although an increase in food intake has been reported in one experiment [28]. The effect of PCPA on water intake remains unclear. Most researchers have found a decrease [6] or no change in drinking [8, 12, 31]. However, hyperdipsia has been observed in one experiment

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([7] cited by [8]). A transient increase in irritability and resistance to handling has often been noticed after PCPA injections [19,41]. The increase in irritability is small and some experimenters have not observed it [5]. Muricide has consistently been observed following PCPA injections [11,35]. The topography of the killing response shown by PCPA-treated rats differs from that shown by natural mouse-killers in that the PCPA-treated rats bite the mouse indiscriminately over the entire body, whereas spontaneous killers bite mainly the cervical region [25]. PCPA injections increase the percentage of males intromitting and ejaculating, provided these males show a low baseline response level [14, 22, 23, 39]. PCPA injections do not increase the number of ejaculations in rats already showing high baseline copulatory levels [47]; however, they do reduce the latency of ejaculation [2,34].

In the present experiment, PCPA injections were given to two groups of rats in order to provide additional evidence for the involvement of 5-HT in the control of behavior. In Group 1 the injection schedule was designed to maintain continuously low levels of 5-HT. In Group 2, the injection schedule was designed to deplete 5-HT, allow it to return to near control levels, and to deplete it again. In both groups measurements on eating, drinking, irritability and copulation were taken before and after PCPA or vehicle injections; measurements on muricide were taken before and after injections in Group 1, but only after injections in Group 2. The multiple measurement approach was used because it provides evidence as to whether some of the behaviors examined may be controlled by common physiological substrates. In addition, this approach helps in assessing the specificity of drug-induced changes. If after a drug injection all behaviors are enhanced or suppressed, it could be suspected that the drug has a general action. However, if after the injection some behaviors change, while others remain unchanged or move in the opposite direction, then this would suggest a possible specific action of the drug. Finally, in the present experiment, where the effects of PCPA on a behavior such as muricide are well documented, the appearance of muricide can be taken as a behavioral indication that the rats are in fact receiving the treatment they are supposed to receive.

METHOD

Animals

Forty-four male Sprague-Dawley rats weighing approximately 500 g (about 6 months old) at the time of the first injection were used. They were naive at the commencement of the experiment and were individually housed in wire mesh cages in a room with a reversed 12:12 hr light/dark cycle. The data from one rat that died prematurely and another that had a brain tumor were excluded from the analysis. Eleven rats were randomly assigned to Group 1, 18 to Group 2, 6 to Vehicle Control Group 1 and 7 to Vehicle Control Group 2. Control rats received vehicle injections at appropriate times. Since behaviorally and biochemically there were no significant differences between the two control groups, their data were pooled.

Food and Water Intake, and Body Weight

Measurements of food and water intake, and body weight were taken every third day from 9 days before to 21 days after the first injection. Each rat was given a weighed quantity of Allied Rat-and-Mouse Kubes. The amount of food given was always in excess of what the rats ate, and at the end of the 3 days the remaining quantity (including any substantial spillage) was weighed. Every third day fresh tap water was provided in 600 ml bottles fitted with tubes that contained steel balls to reduce spillage.

Irritability and Muricide

Irritability scores were obtained on every third day from 9 days before to 21 days after the first injection using the following 3-category scale: (a) biting reaction to a gloved hand that intruded into the cage and pushed the rat against the cage wall, (b) resistance to capture, and (c) vocalization during the preceding two tests. Ratings of 0 (no response), 1 (weak response), or 2 (intense response) were given for each category. All ratings were given by one experimenter. To evaluate muricide in Group 1, 20 min after each irritability test a mouse was placed into the rat's cage for 1 hr, and instances (no latencies) of killing were recorded. For Group 2, muricide was tested similarly but only after the commencement of injections. Rats in Group 2 were not tested for mouse killing prior to the initiation of PCPA injections because it has been suggested that such pretesting may inhibit subsequent development of mouse killing [17].

Copulation

In order to achieve a stable baseline of sexual responding, each rat was given one practice session per week for 6–8 weeks before copulation measurements were commenced. Sexual practice sessions took place during the dark cycle. The rat was placed in a clear acrylic box (40 × 25 × 40 cm) with a wire mesh floor and red-light illumination. After a 5 min adaptation period, a female rat that had been brought into heat with hormonal injections (0.1 mg of estradiol valerate repeated after 2 days, and followed a day later by 1.0 mg of hydroxyprogesterone 5 hr before testing) was introduced into the box. In order to increase the percentage of male rats that would perform sexually, rats that did not copulate within 5–10 min after the introduction of the female were given an occasional tail pinch during the practice sessions. Tail pinches are known to facilitate copulation [9].

After a baseline of sexual responding had been achieved, each rat was given one copulation test per week for 3 weeks, and these three tests provided the preinjection copulation data. Starting 1 week after the first injection, each rat was given one copulation test per week for 3 weeks, and these provided the postinjection data. The three preinjection and three postinjection copulation tests lasted 40 min each, and the following measures were taken: mount latency (mount with pelvic thrusting) and number of mounts per ejaculation; intromission latency and number of intromissions per ejaculation; and ejaculation latency and number of ejaculations during the 40 min test. During the three preinjection and three postinjection tests, the procedure of tail pinching was not employed.

Injection Procedures

DL-PCPA (Sigma Chemical Co.) was prepared as a 100-mg/ml suspension in an isotonic 1% solution of Tween 80 buffered to pH 7.4. Injections were given intraperitonially and a magnetic stirrer was used to agitate the suspension until the moment of each injection. Table 1

TABLE 1

SCHEDULE OF TESTS AND INJECTIONS

Preinjection Period (Days)									Postinjection Period (Days)									
							C											
		F	F	F	F	F	F		F		F		F		F		F	
		W	W	W	W	W	W		W		W		W		W		W	
		Wt	Wt	Wt	Wt	Wt	Wt		Wt		Wt		Wt		Wt		Wt	
		I	I	I	I	I	I		I		I		I		I		I	
C	C	M	M	C M	M	M	M		M	C	M		M		M	C	M	
15 14 13 12 11 10 9	8 7	6 5 4	3 2	1 1 2 3	4 5 6	7 8 9	10 11 12		13	14 15	16 17 18		19 20			21	22	
				*		*			*				*					
				- + +	+						+ + +		+ +					

F = Food.
I = Irritability.
M = Muricide.
C = Copulation.
W = Water.
Wt = Weight.
* = Injections for Group 1 (350 mg/kg PCPA).
+ = Injections for Group 2 (100 mg/kg PCPA).

shows the days on which behavioral testing and injections occurred. When behavioral testing and injections occurred on the same day, injections were always administered after behavioral testing. The first injection was given after measures on eating, drinking, body weight, irritability, muricide, and copulation were taken three times (preinjection baseline data). Preinjection baseline data on muricide were taken only in Group 1.

In Group 1, the injection schedule was designed to maintain low levels of 5-HT continuously. Koe and Weissman [19] reported that a single dose of 316 mg/kg of PCPA maintains brain 5-HT levels below 20% of control values for 6–7 days. Similar PCPA doses deplete NE and DA by about 20% for 5–6 days, but the CA levels are not significantly depleted after this period [13, 19, 26]. In the present experiment four 350 mg/kg PCPA injections were given to rats of Group 1. The injections were separated from each other by 5 day injection free periods.

In Group 2, the injection schedule was designed to deplete 5-HT, to allow it to return to near control levels, and to deplete it again. Drug doses were therefore separated by a 10 day injection free period during which time 5-HT levels should have risen to about 60–70% of control levels [19]. In addition, the dose was changed from a single 350 mg/kg injection in Group 1 to five once-daily 100 mg/kg injections in Group 2. This regime of five injections was chosen so as to minimize depletion of brain CA and to make the procedure more comparable with that used in studies reporting hypersexuality [13].

At the end of the experiment, six irritable mouse killers from Group 2 were injected intraperitonially with 80 mg/kg 5-hydroxytryptophan (5-HTP) in an attempt to block these behaviors by replenishing brain 5-HT levels. DL-5-HTP (Sigma Chemical Co.) was prepared as an 80 mg/ml solution in physiological saline. The rats were tested for irritability and muricide 20 min after the 5-HTP injection.

Assay Procedure

On completion of behavioral testing, the rats were decapitated and, after discarding the pineal gland, their forebrains were dissected by a cut from the rostral border of the superior colliculi dorsally, to the caudal border of the mammillary bodies ventrally [1,15]. Forebrains were frozen on dry ice and kept at −22°C until assayed for 5-HT by a modification [1] of the method of Snyder, Axelrod, and Zweig [37]. The frozen tissue was homogenized in 6 ml of 0.1 NHCl and added to tubes containing 1.5 ml saturated Na_2CO_3, 2.5 ml, 0.5 M borate buffer (pH 11) saturated with n-butanol and NaCl, 8.5 ml of n-butanol, and 2.5 g of NaCl. After shaking for 5 min followed by centrifugation, 5.5 ml of the organic phases were transferred to tubes containing 8 ml of 0.1 M borate buffer (pH 11: previously saturated with NaCl). After shaking for 5 min and centrifuging, 4 ml of the organic phases were transferred to tubes containing 1.5 ml of 0.05 M sodium phosphate buffer (pH 6.5) and 10 ml of heptane. After shaking for 3 min and centrifuging, the aqueous phases were transferred to tubes containing 0.1 ml of 0.1 M ninhydrin solution. After the mixtures were heated in a water bath at 75°C for 30 min and cooled at room temperature for 30 min, their fluorescence at 490 nm (activation 385 nm) was measured in an Aminco-Bowman spectrophotoflurometer. 5-HT concentrations were not corrected for recovery because 5-HT added to tissue is less stable than endogemous 5-HT (Richard Green, personal communication).

This assay gave 5-HT levels for Group 1 and control rats that fell within the range reported by other laboratories [1, 13, 19, 25]. Brains from Group 2 were found to have spuriously high 5-HT values, probably due to interference of PCPA with this assay [3]. In Group 1 the last PCPA injections were given 3 days prior to decapitation, but in Group 2 the PCPA injections were given 2 days prior to decapitation. In order to obtain an estimate of the 5-HT depletion that resulted from Group 2 schedule of injections, 12 naive rats were used and treated as the rats of Group 2 except that they were not tested for copulation and were decapitated 6 days after the last injection. Ten of these rats were injected with PCPA and two with the vehicle. The 10 PCPA-injected rats in this group lost weight and drank more water: in addition four of them killed

mice. Thus, this group behaved similarly to Group 2 (see below); however, the behavioral data from this group were not used in any of the evaluations in the present experiment. It should be emphasized that there is no direct evidence of the extent of 5-HT depletion in Group 2. An indication of 5-HT levels in Group 2 comes from the group of 10 rats which were given the same injections as rats of Group 2, and these levels are relevant to a time 6 days after the last PCPA injection.

Statistical Analysis

On all behaviors, for each rat the scores prior to the first injection were subtracted from the scores after the first injection. The difference scores for the PCPA-injected and control groups were compared with F tests. On muricide, the number of tests on which the rat killed constituted its score; since no control rat killed, one of them was given a score of 0.1 to avoid a S.D. of zero. Pearson's correlation coefficients were calculated between the different variables in the three groups.

TABLE 2

EFFECTS OF PARA-CHLOROPHENYLALANINE ON FOREBRAIN 5-HYDROXYTRYPTAMINE LEVELS

Group	N	5-HT (ng/g) levels ± S.E.	Percent depletion
1	11	109 ± 14	78*
Rats given injections similar to those in Group 2	10	147 ± 15	71*
Control	13	492 ± 11	—

*p 0.001.

RESULTS

5-HT Levels

Table 2 shows the forebrain 5-HT levels and percent depletion for the groups that were assayed. Compared to the control group, Group 1 showed a 78% depletion, $F(1,22) = 289.25$ $p<0.001$, and the group that received PCPA injections similar to those of Group 2 showed a 71% depletion, $F(1,21) = 225.16$, $p<0.001$. 5-HT assays were carried out 3 days after the last 350 mg/kg PCPA injection in Group 1 and 6 days after the last injection in a series of five daily 100 mg/kg injections in the group that received injections similar to those of Group 2. Thus, the depletion data in the two groups are not directly comparable. They do show, however, that in both groups the PCPA injections were effective in depleting forebrain 5-HT.

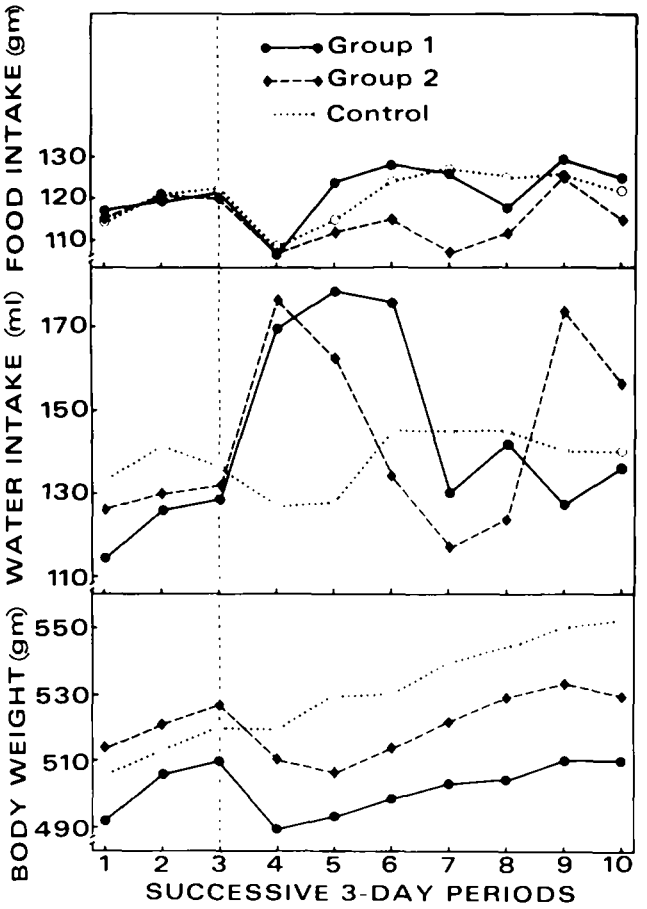

FIG. 1. Mean 3-day food and water intake and mean Day 3 body weight before and after PCPA (Groups 1 & 2) or control injections. (The interrupted line indicates the time of first injection).

Food Intake and Body Weight

A significant decrease in body weight was observed in PCPA Group 1, $F(1,22) = 78.01$, p 0.001, and PCPA

TABLE 3

BEHAVIORAL FINDINGS BEFORE AND AFTER INJECTIONS

Group	N	Mean food intake (in g/day)		Mean water intake (in ml/day)		Mean body weight (g)		Mean irritability score per test		No. of mouse killers		Mean No. of ejaculations per test	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	11	40	41	41	50*	502	501†	0.5	1.5	0	3	2.3	2.6
2	18	40	38*	43	50*	521	521†	0.7	1.9		14†	1.9	2.3
Control	13	40	40	46	46	513	538	0.3	0.6	0	0	1.7	2.4

*$p<0.01$.
†$p<0.001$.

Group 2, $F(1,29) = 49.38$, $p<0.001$ (Fig. 1, Table 3). Body weights of both groups reached preinjection levels at the end of the experiment, 3 weeks after the first injection. Only PCPA Group 2 showed a slight but significant decrease in food intake. $F(1,29) = 8.51$, $p<0.01$ (Fig. 1, Table 3).

Water Intake

After PCPA injections, hyperdipsia appeared in both Group 1, $F(1,22) = 9.14$, $p<0.01$, and Group 2, $F(1,29) = 8.35$, $p<0.01$ (Fig. 1, Table 3). Twenty-four of the 29 PCPA-injected rats in Groups 1 and 2 showed an increase in the mean daily water intake for the 3 weeks after commencement of PCPA injections. the 5 rats that did not show an overall increase in drinking still showed some increase for the first few days after commencement of injections. As shown in Fig. 1, water intake for Group 1 increased by approximately 40% and this effect persisted throughout the 6 days following the first injection and the 3 days following the second injection; injections 3 and 4 produced only minor increase in water intake. For Group 2, during the 3 days following the commencement of the first series of injections water intake increased by 36% and it remained above preinjection baseline values for 6 days after the first injection. Hyperdipsia in Group 2 was reinstated when the PCPA injections were repeated. For the 3 days following the commencement of the second series of injections the rats showed a 34% increase in water intake over baseline intake.

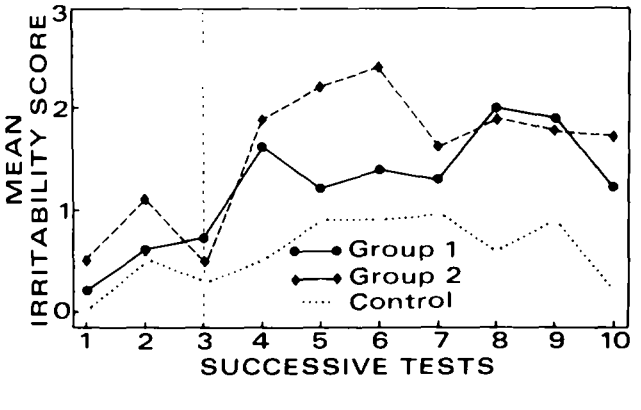

FIG. 2. Mean irritability scores before and after PCPA (Groups 1 & 2) or control injections. (The interrupted line shows the time of first injection).

Irritability

PCPA injections caused a non-significant increase in irritability in both PCPA groups. Eight of the 11 rats in Group 1 showed postinjection increases in resistance to capture, vocalization, and glove biting. Similar changes were observed in 15 of the 18 rats in Group 2 and in 2 of the 13 controls. As seen in Fig. 2, the time course of irritability was not closely associated with the time of PCPA injections.

Of the six irritable killers which received 5-HTP injections (80 mg/kg), five lost their irritability within 20 min of the injections. The rat that remained irritable was one of the two rats which continued killing mice after 5-HTP injections (see below).

TABLE 4

KILLING IN GROUP 2 RATS

Rat	Successive Tests*						
	1	2	3	4	5	6	7
1						K	
2	K	K	K	K	K	K	K
3	K	K	K	K	K	K	K
4	K	K	K	K	K	K	K
5	K	K	K	K	K	K	K
6							
7	K	K	K	K	K	K	K
8	K	K					
9	K	K	K	K		K	K
10							
11	K	K	K	K	K	K	K
12	K	K	K	K	K	K	K
13							
14							K
15	K	K	K			K	K
16							
17	K	K	K	K	K	K	K
18	K	K	K			K	K

K: Kill.
*Test 1 was given 3 days after the first series of PCPA injections commenced and Test 6 was given 3 days after the second series of PCPA injections commenced.

Muricide

None of the 13 control rats killed mice on any test and none of Group 1 rats killed mice on the three preinjection tests. After injection, three Group 1 rats killed mice on at least some of the tests. The 5-HT depletion in the killer rats (79%) was not significantly different from that in the nonkillers (78%).

Group 2 rats were not tested for mouse killing prior to the commencement of PCPA injections. After injection, 14 of the 18 rats (78%) killed mice on at least some of the tests (Table 4) compared with none of the 13 control rats. $F(1,29) = 23.83$, $p<0.001$. Figure 3 shows the percentage of rats in Group 2 killing mice on each test. This percentage declined from 67% to 44% during the period between the two series of PCPA injections when presumably 5-HT levels were nearer to normal. Killers of both groups ate at least part of the mouse on almost all tests.

Intraperitonial administration of 80 mg/kg of 5-HTP at the end of the experiment blocked killing in four of the six injected mouse killers. The 5-HTP injected rats did not appear somnolescent and actively investigated the mice.

Copulation

As Fig. 4 and Table 3 indicate, there were no significant differences between PCPA-injected and vehicle-injected groups in their ejaculatory performance before and after injection. All groups showed a slight increase in the number of ejaculations on successive tests indicating that a stable baseline of ejaculatory performance had not been achieved in the 2 months of weekly preinjection practice trials. The maximum number of ejaculations shown by any rat on a single 40 min test was five.

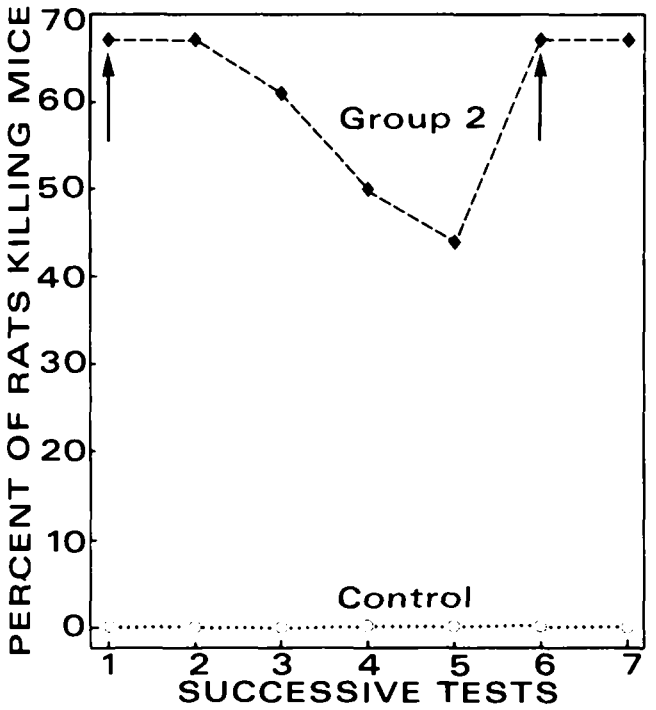

FIG. 3. Percentage of rats in Group 2 and control group killing mice on each test. (The left and right arrows indicate the first test after the commencement of the first and second series of injections, respectively).

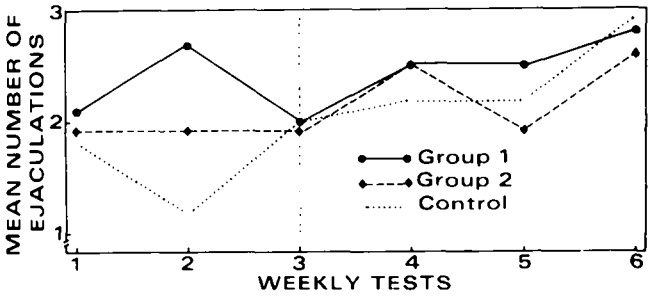

FIG. 4. Mean number of ejaculations before and after PCPA injections. (The interrupted line shows the time of first injection).

With regard to other measures of copulatory performance, all groups showed a large variability in the number of mounts, intromissions, and latencies, and there were no significant differences in these measures between the PCPA-injected and control groups.

DISCUSSION

Food Intake and Body Weight

Both PCPA groups showed a significant decrease in body weight, but only Group 2 showed a decrease in food intake. The small decrease in food intake observed in Group 2 was not commensurate to the drastic fall in body weight. The absence of a substantial decrease in food intake was not due to spillage because spillage was always weighed and included in the calculation of food intake. The partial dissociation of food intake from body weight suggests that PCPA injections produce metabolic changes. This is consistent with data indicating 5-HT involvement in the control of the cardiovascular, respiratory, endocrine, and arousal systems [30].

Water Intake

In both groups of the present study hyperdipsia appeared in almost all rats injected with PCPA. In Group 2, where the series of PCPA injections were separated by a 10 day injection free period, hyperdipsia appeared, disappeared, and reappeared in association with injections. Published studies report either a decrease or no change in drinking following PCPA injections [6,12]. While Borbély *et al.* [6] obtained an overall decrease in water intake following a 300 mg/kg PCPA injection, three of their rats were hyperdipsic on the third day after injection. Brody [8] did not obtain changes in water intake following five daily 100 mg/kg injections of PCPA. However, he referred to a study in which he obtained hyperdipsia following a 300 mg/kg injection [7]. Results from the present experiment are consistent with Brody's unpublished observations.

The question arises as to whether central 5-HT depletion was responsible for the hyperdipsia observed in the present study. Support for 5-HT involvement in hyperdipsia comes from Group 2 data where hyperdipsia disappeared a few days after cessation of PCPA injections, when 5-HT levels were probably returning to normal [19], and reappeared after reinitiation of the injections. 5-HT involvement in hyperdipsia is also suggested by studies where lesions of the raphe nuclei are reported to produce hyperdipsia ([10, 21, 40] but see [38]). On the other hand, in Group 1 5-HT levels were probably at a continuously low level for several weeks, yet hyperdipsia was evident only for the first 9 days. While the data from Group 1 could be construed as contraindicating a serotonergic explanation of hyperdipsia, the recovery of apparently normal drinking while 5-HT was presumably still depleted might reflect a compensatory increase in 5-HT receptor sensitivity. Drug-induced supersensitivity is well documented in catecholaminergic neurons [27], and there is some evidence that it occurs in indoleaminergic neurons as well [18].

While the above considerations suggest that 5-HT depletion may be responsible for the observed hyperdipsia, CA depletion should not be overlooked. CA levels were not measured in the present experiment, but from the work of others [19,26] it is expected that CA levels in, e.g., Group 2 should have been depleted by about 20% and that they should have returned to normal during the 10 day injection free interval. Thus, the time course of the CA depletion parallels that of hyperdipsia. The effect of PCPA on 5-HT is, of course, much greater than its effect on the CA, but, as Welch and Welch [45,46] have argued, it is possible that the small CA depletion is functionally important.

Irritability

Both PCPA groups in the present experiment showed a slight increase in irritability, but this increase did not reach significance. Two control rats showed increased irritability as well, indicating that some of the increase was due to nonspecific effects of the injection procedure. The irritability scale used in the present experiment did not include fine tests such as reactions to a tap on the back or responses to a discrete air puff delivered through a syringe to the rat's body [42]. These tests might have detected subtle differ-

TABLE 5

CORRELATIONS

	PCPA GROUP 1 N = 11	PCPA GROUP 2 N = 18	CONTROL N = 13
5-HT and irritability	−.47		−.50
5-HT and weight	.23		−.56
5-HT and food	−.24		−.20
5-HT and water	−.02		−.24
5-HT and muricide	.16		.07
5-HT and copulation	.42		.48
Irritability and weight	.40	−.15	.13
Irritability and food	−.15	.28	−.14
Irritability and water	.05	.10	−.16
Irritability and muricide	−.28	.16	−.10
Irritability and copulation	−.30	−.02	−.55
Weight and food	−.17	.47	.55
Weight and water	.26	.32	.44
Weight and muricide	−.11	−.03	−.24
Weight and copulation	.52	.20	−.22
Food and water	.33	−.02	.26
Food and muricide	.56	−.19	.00
Food and copulation	−.21	.09	.38
Water and muricide	−.07	.21	.05
Water and copulation	.23	.27	−.08
Muricide and copulation	−.25	−.11	.12

ences between experimental and control groups. However, the present data are consistent with reports of minor increases in irritability after PCPA injections [19].

An injection of 5-HTP abolished irritability in five of the 6 rats that became irritable after PCPA injections. The rats did not appear somnolescent at the time of testing. However, it is not clear whether the 5-HTP injections specifically suppressed irritability or had a general suppressive effect.

Muricide

The present data confirm earlier reports of increased muricide after PCPA injections and give some support to the notion that brain 5-HT may normally function to inhibit mouse killing. In Group 2 of the present study 78% of the rats killed mice. More of these rats killed mice during the two PCPA series of injections than during the injection free interval when 5-HT levels were presumably closer to normal (about 60 70% of normal; [19]). In addition, administration of 5-HTP blocked killing in four of the six rats that killed after PCPA injections. Electrolytic lesions of the raphe nuclei or injections of 5, 7-dihydroxytryptamine into the ascending 5-HT projection do not reliably induce muricide unless the 5-HT depletion they cause reaches 70 90% [20, 33, 36, 44]. Since in achieving high 5-HT depletion, lesions and injections affect other systems, such as the CA, in addition to 5-HT, it is still not clear whether 5-HT depletion alone is sufficient to induce killing.

The low percentage of mouse killers in Group 1 (27%) relative to that in Group 2 (78%) may have been due either to the dose schedule or to some inhibition resulting from pretesting. The present study provides no evidence as to the relative importance of these two factors, but it has been shown that in certain other experimental conditions pretesting inhibits subsequent development of muricide. Thus, Paul [32] found that prior exposure to mice reduces the number of rats that are induced to kill by food deprivation. Marks, O'Brien, and Paxinos (unpublished observations) injected two groups of rats with 8 μg/4 μl of 5,7-dihydroxytryptamine into the ascending 5-HT projection after having pretested one of the groups with mice. In the pretested group only 1 of its 15 rats killed mice postoperatively, but in the group that was tested only postoperatively, 9 of its 16 rats killed. Myers [29] observed that if a rat does not kill, on successive sessions its responding to the mouse (e.g., smelling, grooming, and retrieving) decreases, and he suggested that this habituation may account for the fact that the probability that a rat will begin killing declines in successive tests. Myers' considerations would suggest that habituation resulting from pretesting attenuates increases in muricidal tendencies that may follow an experimental manipulation.

Copulation

In the present experiment the preinjection copulation practice trials produced relatively high baseline response levels, and PCPA injections did not enhance this performance. Other experimenters have found that PCPA injections enhance copulation in males showing low baseline response levels [39], but that these injections have either no effect ([47 McDonald, Doughty and Barfield cited by [4]) or a minor effect [34] on sexually experienced males.

Correlations

In the present experiment, PCPA injections produced forebrain 5-HT depletion, hypophagia, hyperdipsia, weight loss, and muricide. There were no significant correlations

between the different effects (Table 5), and this could be taken to suggest that PCPA produces its behavioral effects by other means in addition to 5-HT depletion. However, in view of the small sample sizes (N = 11, 18, 13) and the absence of 5-HT measurements from Group 2, this suggestion should be regarded as tentative.

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