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para-Chloro-D,L-phenylalanine Induced Filicidal Behavior in the Female Rat

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COPENHAVER, J. H., R. L. SCHALOCK AND M. J. CARVER. *para-Chloro-D,L-phenylalanine induced filicidal behavior.* PHARMAC. BIOCHEM. BEHAV. 8(3) 263–270, 1978. – The administration of para-chloro-D,L-phenylalanine (PCPA) produces a high incidence of aggressive (filicidal) behavior in pre-, postpartum, and nulliparious rodents. PCPA inhibits brain tryptophan 5-monooxygenase and can produce a reduction in whole brain serotonin. Apparently PCPA mediates the release of a natural latent aggressive tendency which is potentiated by the interference in, or reduction of, a suppressing system governed primarily by serotonin. Latency of attack, intensity phases, and characteristics of the filicidal behavior were found to vary inversely with brain serotonin content, and be reversed or eliminated by replacement of serotonin i.e., via 5-hydroxytryptophan, serotonin's immediate precursor. Although aggressive tendencies were in evidence prior to filicide, filicide became evident once the apparently minimal whole brain level of serotonin reached ca 0.10 μg/g. Neither the parturition process nor severe food deprivation are strong causative factors in the precipitation of filicidal behavior. Since not all animals become filicidal, other behavioral and/or biological variables must be involved in the mediation of this aggressive phenomenon.

para-Chloro-D,L-phenylalanine Filicidal behavior Aggression

DURING the process of developing a murine model of maternal hyperphenylalaninemia, we observed that pregnant animals treated with phenylalanine and para-chlorophenylalanine (PCPA) delivered their offspring, but destroyed them during, or shortly after, the parturition process [3].

Although it was our intent to employ PCPA to inhibit in vivo the maternal animal's hepatic phenylalanine 4-monooxygenase (E.C. 1.14.16.1), we were also inhibiting the maternal animal's brain tryptophan 5-monooxygenase (E.C. 1.14.16.4) and concomitantly producing a reduction in brain serotonin [8, 9, 10].

Serotonin has been implicated in muricidal (mouse-killing) behavior in rats with substantial depletion of forebrain serotonin levels [17]. Additional evidence has been presented which also demonstrates that serotonin depletion results in muricidal behavior [11]. Conversely, others have found that muricidal behavior can be suppressed by treatment of the animal with 5-hydroxytryptophan, the immediate precursor to serotonin [5]. Induction of muricidal behavior in PCPA treated olfactory bulb-lesioned rats has led investigators to conclude that the olfactory nucleus is involved in the inhibition of predatory aggression [5].

Conversely, PCPA has been found to antagonize, or have no effect, on other types of aggressive behavior. For example, the fighting behavior of shock-induced rats appears to be uninfluenced by PCPA and aggressivity in induced isolation in mice was diminished following the administration of PCPA [1,20]. Several groups of investigators have found that PCPA treated rats had unaltered fighting behavior following foot shock [1, 2, 12], while others have reported increased shock-elicited aggression following PCPA administration [1, 2, 7, 12]. PCPA has been shown to attenuate aggressiveness and irritability of rats with septal lesions [6].

The present investigation dealt with the induction of a killing behavior, referred to as filicide, in the pre-, post-partum, and nulliparious adult female rat treated with PCPA. We are unaware of any reports indicating that PCPA is capable of precipitating a maternal pup-killing behavioral state (filicide) in rats or other animals.

METHOD

Animals

Population and Strain. Adult Sprague-Dawley female and male rats were obtained from the Small Animal Supply Company (SASCO), Omaha, NE. The animals were provided food (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) and water ad lib. Adult animals were housed in metal cages (9 x 14 x 20 inches) in air conditioned (23°) quarters with light (0600 to 1800 hr)–dark condition.

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Pregnancies. Pregnancies were established from the above population. The day the animal was found to be sperm positive was considered day zero gestation (GD 0). Unless the pups were used as filicide stimulus objects the maternal animals and offspring were not disturbed until the seventh postpartum day (PPD 7). If the pups were not used, they were sexed on PPD 7 and, if possible, four male and four females were retained in the litter. Pups in excess of eight were removed and destroyed.

Chemicals

L-phenylalanine (PA), para-chloro-D,L-phenylalanine (PCPA), 5-hydroxy-L-tryptophan (5-HTP) and 5-hydroxy-tryptamine (serotonin) creatinine salt (5-HT·CS) were purchased from Sigma Chemical Company, St. Louis, MO. All chemicals were suspended in a vehicle of carboxy-methylcellulose (CMC, 10 mg/ml). PCPA, PA and CMC were injected subcutaneously (18 gauge needle) in the nape of the neck. 5-HTP and 5-HT· CS were injected intra-peritoneally.

Animal Designation, Preparation, Terms and Nomenclature

Prepartum maternal animals. A total of 39 animals received PCPA (1.0 mMole/kg) on GD 14, 17 and PA (1.0 mMole/kg) on GD 14–20. Four animals received PA, or PCPA alone, and five animals received CMC. Pregnant animals were given PA at 0800 to 0900 hr, or four hr after PCPA if both compounds were employed, with CMC being administered by the same route. With the above exceptions, the regimen of PA/PCPA was given to all animals, as it was found to be one of the milder regimens which produced a hyperphenylalaninemia condition in pregnant animals. Varying doses of 5-HTP (n = 23, 10–200 mg/kg) were given on either GD 17, 20, or 19, 20 to those animals receiving PA/PCPA. Also, four animals received less than ten mg/kg of 5-HTP. Brain serotonin levels were not determined on any of those animals. Prepartum animals, administered PCPA, PCPA/PA, or PA, were considered filicidal if the entire litter was destroyed during or following parturition (PPD 1). The behavior was considered to be cannibalistic in nature if one or more of the pups was consumed by the maternal animal. If this occurred the adult was removed from the study. Nine animals were removed from this study either because of cannibalistic behavior or filicidal behavior resulting in only a portion of the litter being destroyed.

Postpartum maternal animals. Primiparious animals were not interferred with prior to delivery. On the first day following parturition (PPD 1), the maternal animal was weighed and for three days (postpartum days, PPD 1–3), the adult animal received injections of PCPA (1.5 mMole/kg) or CMC (control). Daily thereafter (PPD 1–12), the adult animal was removed from her litter and placed in a test cage for up to ten min with either a surrogate and/or her own pup. Latency of the attack was recorded and the maternal animal returned to her own litter and cage. Seven similarly treated animals were sacrificed on consecutive days (PPD 1–12), and the whole brain level of serotonin was determined.

Nulliparious (nonmaternal) animals. Because of the logistics, and assumed accerbating effects of the parturition process upon the filicidal studies, nulliparious adult females were employed as potential filicidal animals if the animal was over 60 days of age and weighed in excess of 180 g. All nulliparious animals were tested for spontaneous filicidal behavior on Day one (D 1). An adult female was placed in an opaque plastic testing cage (7 × 13 × 14 inches, covered with about one inch of litter, Bed-O-Cobs, Anderson Cobb Mills, Inc., Maumee, OH), for five min acclimation period after which a 3–7 day old pup, of either sex, was placed into the testing chamber at a point most distant from the adult. If the adult failed to attack the pup, the adult was removed, weighed, and given PCPA, (2.0 mMole/kg) between 0800 to 0900 hr on D 1 and retested at 1300 to 1500 hr. If the animal attacked the pup it was considered spontaneously filicidal and removed from this portion of the study. The remaining nonspontaneous nonfilicidal animals were administered PCPA at 0800 to 0900 hr on D 2–3, and tested for filicidal behavior at 1300 to 1500 hr on D 2 through 5.

Twenty-five animals and five CMC control animals were selected and divided into subgroups according to the criteria: PCPA inducible (n = 14, defined as an animal which attains a filicide index $\geqslant 85$ on Day 4 following three consecutive injections of PCPA); PCPA semi-inducible (n = 5, defined as an animal which does not attain a filicide index $\geqslant 85$ on Day 4, and/or exhibit consistent filicidal tendencies during, or after, three consecutive days of PCPA); and PCPA non-inducible (n = 6, defined as an animal which fails to kill a pup on any of the five days during, or after, administration of PCPA). The PCPA inducible animals were further divided into three groups of five, five, and four animals, and given PCPA, PCPA and 5-HTP, or PCPA and 5-HT·CS respectively. These animals were weighed daily, and activity measurements taken prior to, and at three hr after, an injection of 5-HTP or 5-HT·CS at 0100 hr on D 4–5.

Filicidal Measurements and Index

An index of the animal's filicidal propensity was estimated by subtracting the latency (L) of the attack from 600 sec, the time of the total test period, and dividing this value by 600 sec to obtain a ratio. The ratio $\times 10^{+2}$ (filicide index, FI) could range therefore from near zero (non-filicidal), or zero if the animal failed to attack during the 600 sec test period, to approximately one hundred (strongly filicidal).

Activity Measurements

Activity levels consisted of counting the number of grid lines (6 × 6 inch squares, possible total of 32 lines) crossed in three min by the animal.

Serotonin Determinations

Brain serotonin levels were determined by the methods of Wise from whole brain, [21].

RESULTS

Prepartum Maternal Animals

All prepartum animals (n = 39) treated with PCPA alone on GD 14 and 17, or in combination with PA on GD 14–20 destroyed all or a portion of their offspring during or within 24 hr following parturition. Animals receiving PA alone, or CMC, delivered and maintained their offspring through PPD 8 or weaning age.

TABLE 1

FILICIDE PROBABILITY, FILICIDE INDEX AND WHOLE BRAIN SEROTONIN CONTENT IN POSTPARTUM LACTATING FEMALE ANIMALS RECEIVING MULTIPLE INJECTIONS OF PARA-CHLORO-D,L-PHENYLALANINE*

PPD	PCPA	Filicide Test	Filicide Probability	Mean Filicide Index ± SEM		Whole Brain Serotonin Content (μg/g) ± SEM†	
(N = 5)	–	–	0.00	00	–	0.54	0.03
1	+	+	0.00	00	–	0.20	0.02
2	+	+	0.00	00	–	0.15	0.01
3	+	+	0.50	67	5	0.07	0.01
4	–	+	0.67	85	9	0.06	0.02
5	–	+	0.95	94	8	0.06	0.01
6	–	+	0.83	96	9	0.08	0.02
7	–	+	0.83	94	5	0.13	0.03
8	–	+	0.80	83	12	0.11	0.01
9	–	+	0.40	08	15	0.26	0.08
10	–	+	0.00	00	–	0.18	0.02
11	–	+	0.00	00	–	0.21	0.09
12	–	+	0.00	00	–	0.35	0.07

*N = 24

†Mean whole brain serotonin content of seven postpartum animals at each PPD. Animals analyzed for serotonin were not the same animals as those upon whom the filicide index was determined.

If an adult animal was treated with ten or more mg of 5-HTP prior to parturition, the animal usually delivered normally and maintained the litter through PPD 8, or through the weaning stage. Animals treated with 5-HTP (n = 5, 100–200 mg ca. 0.5 to 1.0 mMole/kg) on GD 17, 20 or 19, 20 produced stillborn animals, but did not cannibalize the offspring. Animals treated with 5-HTP (n = 2, 50 mg) on GD 19, 20, produced stillborn pups; but if an animal (n = 2) was treated on GD 17, 20, it produced apparently normal litters with no maternal filicide. Animals treated with 5-HTP (n = 14, 10–25 mg) on GD 17, 20 or 19, 20 produced normal offspring, which were maintained by the maternal animal through PPD 8 or weaning age. Four animals treated with less than ten mg produced apparently normal offspring, but filicidal behavior remained prominent during the immediate postpartum period.

Postpartum Maternal Animals

Filicide propensity towards natural offspring, filicide index, whole brain serotonin. Twenty-four nursing animals were administered PCPA (1.5 mMole/kg) on PPD 1–3. Testing for filicidal behavior on surrogate pups began on PPD 1 and continued until PPD 12 (Table 1). Filicide probability increased from 0 on the second day (PPD 2) to 50 by the third day (PPD 3). At that time, whole brain serotonin content had decreased to approximately 13% that found in normal brain. The probability of a nursing maternal animal attacking a surrogate pup had reached 67 or greater by PPD 4 and continued to increase until PPD 8. On about PPD 7–8, whole brain levels of serotonin had increased some two-fold above minimally achieved levels. Normal levels of brain serotonin were not reattained by PPD 12, but were well above the apparently critical level necessary to impede or extinguish the filicidal behavior. In general, filicide was inversely related to whole brain levels of serotonin.

Behavioral changes in maternal filicide – natural offspring. Next, the behavioral characteristics of the maternal animal towards their natural offspring were examined over three arbitrarily defined phases of prefilicidal, filicidal, and postfilicidal behavior. Twenty nursing maternal animals were administered either PCPA (1.5 mg/kg) or CMC on PPD 1–3. On PPD 1, each maternal animal's litter was divided in half, one half remaining with the maternal animal, while the other half was transferred to a surrogate maternal animal. Pups were marked by clipping the tail. Testing began on PPD 1 and continued until PPD 13 (Table 2).

All animals had an increased sniffing behavior during the prefilicidal and filicidal phases. No differences in sniffing behavior towards the natural offspring were evident between CMC or PCPA treated animals. Mauling behavior and emotionality were found to be significantly elevated in PCPA animals during the filicidal phase. The PCPA treated maternal animals preferentially attacked a surrogate pup first over their own on 67% of the trials. Apart from this, the adult did not respond differently to her own as opposed to a surrogate pup.

Nulliparious Animals

Spontaneous filicide. Once the filicidal behavior had been established as not being primarily related to the

TABLE 2

BEHAVIORAL CHANGES DURING DEVELOPMENTAL PHASES IN MATERNAL FILICIDE IN POSTPARTUM NURSING FEMALE ANIMALS RECEIVING MULTIPLE INJECTIONS OF PARA-CHLORO-D,L-PHENYLALANINE, NATURAL AND SURROGATE OFFSPRING

Behavioral Measurement and Experimental Conditions	Offspring Designation	Prefilicidal (PPD 1–2)	Filicidal (PPD 3–10)	Postfilicidal (PPD 11–13)	Total (PPD 1–13)
Sniffs*					
PCPA (n = 10)	Natural	0.50 ± 0.1	0.68 ± 0.13	0.18 ± 0.19	0.45 ± 0.14
	Surrogate	0.45 ± 0.2	0.62 ± 0.20	0.11 ± 0.12	0.39 ± 0.17
CMC-Control (n = 10)	Natural	0.50 ± 0.1	0.47 ± 0.04	0.17 ± 0.20	0.41 ± 0.11
	Surrogate	0.45 ± 0.1	0.67 ± 0.05	0.20 ± 0.24	0.44 ± 0.13
Mauls†					
PCPA	Natural	0.00 ± 0.00	0.48 ± 0.25	0.07 ± 0.08	0.18 ± 0.11
	Surrogate	0.01 ± 0.01	0.54 ± 0.18	0.03 ± 0.01	0.19 ± 0.07
Control	Natural	0.07 ± 0.05	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
	Surrogate	0.04 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	0.02 ± 0.01
Emotionality ‡					
PCPA		1.5 ± 0.02	3.25 ± 0.18	1.75 ± 0.14	2.17 ± 0.11
Control		1.3 ± 0.03	1.62 ± 0.09	1.88 ± 0.21	2.03 ± 0.11
Filicide §					
PCPA	Natural	None	33%	None	33%
	Surrogate	None	67%	None	67%
Control	Natural	None	None	None	None
	Surrogate	None	None	None	None

*Sniffs were defined as female touching pup with nose or bringing nose within approximately 2.5 cm of the pup and expressed as a mean sniffs/minutes ± SEM.

†Mauls were defined as touching, moving or manipulating pup with female's front paws.

‡Expressed as mean fecal boli per trial.

§Expressed as a percentage of time pup was killed first by PCPA or CMC treated animal.

parturition process, we examined the population and behavioral characteristics of a large group (n = 114) of nulliparious animals. Sever percent of this population was found to be spontaneously filicidal i.e., attacked a pup prior to PCPA administration. Six of eight animals found to be spontaneously filicidal were tested for enduring spontaneous filicidal behavior. Of the six animals, three were filicidal on the following day, and two were filicidal on Day 3. Only one attack was observed during a second (8–10 days past the initial tests D 1, 2, 3) or third (15–17 days) trial period. If the original six attacks on Day 1 are ignored, six out of 36 trials resulted in spontaneous filicide with four of the six animals repeating their spontaneous killing behavior.

PCPA inducible filicide. Eighty-four percent (n = 96) of the population (n = 114) were filicidal on the third day. Ninety-one % of these animals had a FI≥50, 74% had a FI≥85, and 66% had a FI≥90 on Day 4.

PCPA non-inducible filicide. Nine percent (n = 10) of the total population were non-filicidal i.e., PCPA was not instrumental in producing any filicidal behavior during the three preparation days or on Days 4 and 5.

PCPA semi-inducible filicide. Animals failing to reach a FI⩾85 on Day 4 and/or exhibit consistent filicidal tendencies during or after the three days of PCPA administration represented 26% (n = 25) of the PCPA inducible filicidal population (n = 96).

Effects of chronic injections of PCPA. Upon: *(a) Non-inducible animals.* Six additional PCPA non-inducible filicide animals were added to the ten non-inducible animals from the original population, and the PCPA regimen repeated for three consecutive days during week two (Days 8–10) and three (Days 15–17). None of the non-inducible animals killed during the second week. Three of the sixteen non-inducible animals killed at least once during the third week (Days 15–19) or a total of three filicidal occurrences out of a total of 240 trials. *(b) Semi-inducible animals.* All of the five semi-inducible animals became filicidal during a second (Days 8–12, 15 occurrences out of 25 trials) treatment intervals, exhibited the same type of filicidal behavior observed in filicide inducible animals, and had similar FI values as inducible animals. Only one of the semi-inducible animals remained relatively non-filicidal during the third week. *(c) Inducible filicide animals.* A group of seven filicidal animals which achieved a FI⩾85 on Day 4 (mean ± SEM, 95 ± 2, Day 5, 95 ±2) of the first week continued to be strongly filicidal during the second (Day 11, 95 ± 2, Day 12, 89 ± 5, 34 out of 35 trials) and third (Day 18, 92 ± 2, Day 19, 79 ± 13, 24 out of 35 trials) week.

Anorexic Effects of PCPA and Relationship to Filicide

As we reported previously PCPA exerts an anorexic effect when administered to animals [4]. To negate the possibility that anorexia was a direct course of filicide, normal primiparious animals were deprived of food, but not water, and compared to animals undergoing acute (Days 1–3) or chronic (Days 1–3, 8–10, 15–17) PCPA regimens.

Normal primiparious postpartum nursing maternal animals – food deprivation. The loss in adult body weight was quite severe through the Days 1–3 interval for all food deprived animals. If an adult nursing animal began its deprivation regimen when the litter had reached a latter stage in development (e.g., PPD 6–13 or 12–19), the loss in body weight was even more severe (n = 2, 16.9–17.8%, and n = 2, 22.4–22.9% loss respectively) when compared to those animals who began the starvation regimen on PPD 2 (n = 2, 11.4–13.5% loss) which was undoubtly due to the larger nursing demands placed on the maternal animal by the pups. All food deprived animals maintained their offspring intact through the first five days of food deprivation, yet none of the six animals were filicidal or cannibalistic during this interval. Very significant decreases in maternal body weight occurred by the fifth day of deprivation in all animals. Animals deprived from PPD 2–9 had lost some 20% of their initial body weight, and animals deprived PPD 6–13, and 12–19 had lost some 28 and 35% of their initial body weight, respectively. On or about the 6–8th day, pups began to die from malnutrition, and in some litters, the adult animal began to cannibalize the dead pups. Of the four animals which lost pups; two cannibalized a portion or all of the dead pups, one did not cannibalize any of the pups, and one pup appeared to have been attacked by its maternal animal as evidenced by slash marks upon the pup's body.

Postpartum nursing animals – PCPA. The body weight of postpartum lactating inducible (n = 18) and non-inducible (n = 2) animals were significantly reduced during the period (PPD 4–6, and 7–9) as compared to control animals. However, both inducible and non-inducible animals continued to lose weight during the initial recovery interval (PPD 8–12). As a group, inducible animals suffered slightly more body weight loss over Days 1–3, 4–6, 7–9, and 10–12, when compared to non-inducible animals (i.e., 3.0 vs 3.0, 8.7 vs 11.8, 8.0 vs 12.5, and 10.0 vs 14.6% respectively). Control animals maintained their initial body weights, or increased slightly, during the same interval.

Nulliparious animals – PCPA. The mean body weight changes in non-inducible (n = 16), semi-inducible (n = 5) and inducible (n = 7) animals were not found to be significantly different; but both non-inducible and inducible groups were more severely affected by the PCPA treatment during 1–3, (7.6, 7.6 vs 5.8%), 1–5 (16.2, 14.0 vs 9.6%) and 8–12 (11.7, 12.9 vs 4.1%) day intervals than semi-inducible animals, respectively. Although not as severely affected by the PCPA regimen during the Days 1–5 interval, semi-inducible animals tended to recover more slowly following the initial Days 1–3 treatment with PCPA, but the same animals regained body weight more rapidly during the Days 8–12 and 8–15 intervals. All three groups reacted similarly to PCPA during the Days 15–19 interval with losses of 10.4 to 15.6%. Each of the three groups had regained, or nearly regained, their original body weights by the 22nd day. As measured by loss in body weight, filicidal animals were more sensitive to PCPA than semi-inducible or non-inducible animals.

Anorexic effects – comparison of food deprived, postpartum nursing and nulliparious animals. There was significantly more weight loss in food deprived maternal nursing animals than in nulliparious or postpartum nursing animals treated with PCPA. Although not strictly comparable, because of the different PCPA mMole/kg regimens (1.0 vs 1.5 vs 2.0), all treated animals underwent significant amounts of body weight loss during the PCPA treatment or deprivation regimens. Significantly, none of the food deprived animals killed their offspring, although deaths of a portion of the pups did occur primarily due to malnutrition.

Filicidal Behavior, Whole Brain Serotonin, and Activity of Non-Maternal and Deprived Nursing Animals

By definition, to be categorized as PCPA inducible animal, an animal had to achieve a FI⩾85. At all four test times (Day 4 hour zero, etc., 4–0′, 4–3′, 5–0′, 5–3′), PCPA inducible animals had a FI⩾85 with small standard deviations, although there was a slight reduction and increase in variability in the filicide propensity of animals examined on 5–0′ and 5–3′ (Table 3).

PCPA semi-inducible animals had a much reduced and highly variable mean FI on 4–0′ (23 ± 29) and 5–0′ (29 ± 30). Non-inducible animals demonstrated no filicidal tendencies on Day 4 or 5. Filicidal animals, employed as animals for 5-HTP or 5-HT·CS administration regimens, had approximately the same FI value on 4–0′ as controls (PCPA inducible animals) i.e., 98 and 96; but the FIs were reduced some 60 to 100%, at 4–3′ following the administration of the respective indole derivatives. The 5-HTP treated animals' FI had increased from 40 to 81 by 5–0′, and responded again to 5-HTP at 5–3′ as they had at 4–3′. The 5-HT·CS treated animals had a much reduced

TABLE 3

EFFECT OF PARA-CHLORO-D,L-PHENYLALANINE ON FILICIDAL BEHAVIOR, ACTIVITY, AND WHOLE BRAIN SEROTONIN IN NULLIPARIOUS AND DEPRIVED FEMALE ANIMALS

Treatment Regimen	Filicidal Behavior Index*				Activity Levels				Serotonin† Mean and Range
	Day 4		Day 5		Day 4		Day 5		
	Hour		Hour		Hour		Hour		
	0	3	0	3	0	3	0	3	
CMC Control (N = 5)	0	0	0	0	20 ± 18‡	–	15 ± 7	–	54 (42–60)
Food Deprivation (N = 6)	0	0	0	0	–	–	–	–	57 (52–61)
PCPA Inducible (N = 5)	97 ± 1	99 ± 1	97 ± 3	95 ± 3	16 ± 12	15 ± 11	52 ± 37	59 ± 26	4 (2–6)
PCPA Semi-Inducible (N = 5)	23 ± 29	–	29 ± 30	–	23 ± 14	–	–	–	5 (5–6)
PCPA Non-Inducible (N = 6)	0	0	0	0	35 ± 10	–	17 ± 7	–	5 (3–7)
5-HTP‖ (N = 5)	98 ± 4	40 ± 34☆	81 ± 17	16 ± 34★	21 ± 9	7 ± 6	10 ± 9	7 ± 5	14 (9–19)
5-HT·CS (N = 4)‖	96 ± 2	0★	14 ± 24★	0★	24 ± 9	5 ± 2	11 ± 8	6 ± 8	35 (32–39)

*See text for definition.

†All serotonin values were obtained at Day 5 Hour 3 (5–3′) and are expressed as μg/g wet brain tissue.

‡Standard Deviation. Significant difference between Day 4–0′ or 5–0′, ☆$p<0.01$, ★$p<0.05$.

§Not determined indicated by a dash.

‖5-Hydroxytryptophan (5-HTP) and serotonin creatinine sulfate salt (5-HT·CS) were administered at dosages of 0.4 mMole/kg b. wt. intraperitoneally at 4–0′ and 5–0′.

FI on 5–0′ as compared to 4–0′ and were non-filicidal on 5–3′. Activity measures did not differ either across days or among groups except in those animals receiving 5-HT·CS. Inducible animals tended to be more active at 5–0′ than at 4–0′, while the reverse was true for non-inducible animals.

DISCUSSION

In general, the present study demonstrates that PCPA produces a time-dependent filicidal behavior i.e., a female animal becomes aggressive towards its own or surrogate pups, while the more natural behavior, as suggested by Moyer, is characterized by an attack by the maternal animal on an intruder or threatening agent [14]. Although filicidal behavior lasts about seven days, the height of the aggressive state is generally around the fourth and fifth days following the initiation of PCPA administration. The aggressive state during this time is very consistent as filicidal females will repeatedly attack, and kill, viable pups for up to thirty, consecutive times, allowing one min rests between kills. This condition was found in a high percentage of pre- and postpartum and nulliparious animals, and presumably, is the result of functionally lowered brain serotonin content. The latency, intensity phases, and characteristics of the filicidal behavior vary inversely with the level of brain serotonin. Therefore, it is reasonable to assume that the serotonergic system is involved in the normal suppression of filicidal behavior. This contention is supported as the administration of 5-hydroxytryptophan, serotonin's precursor, reduced but did not extinguish the filicidal behavior in all cases within three hr after its administration. Furthermore, the filicidal behavior, as depicted by the FI, returned to the level of the previous day. Although a large amount of 5-hydroxytryptophan was given it was apparently not sufficient to completely replace the endogenous depleted 5-hydroxytryptophan. A large amount of serotonin may have been produced, but, only a portion of the newly formed serotonin apparently was utilized i.e., the

remainder being converted to 5-HIAA, or sequestered so rapidly as not to allow the postsynaptic sites to be saturated. This conjecture appears to be borne out as the total brain serotonin level of animals examined, remained at the apparently minimal level whereby filicide tendencies occur i.e., 10–12 μg of 5-HT/g brain.

Serotonin administered as the creatinine sulfate salt extinguished the filicidal behavior, but the dosage employed was so excessive that the animals were immobilized, invalidating any meaningful conclusion with respect to filicidal behavior. Interestingly, the brains of animals analyzed at 5–3', after receiving serotonin, were some seven-fold above minimally achieved levels. Since the filicide propensity had decreased by some 85% at 5–0', this finding suggests that a certain amount of serotonin had crossed the blood brain barrier and had been accumulated, and retained in sufficient quantities to reduce the animal's aggressive tendencies. Immobilization was not an inhibiting factor at 5–0' as the animals had regained their mobility and returned to a normal condition. Although transport apparently occurred following the administration of serotonin·CS, serotonin does not easily cross the blood brain barrier, while conversely 5-hydroxytryptophan can easily enter the brain [18].

Although all PCPA inducible animals were found to have severely depleted brain serotonin levels as were PCPA semi- and non-inducible animals, PCPA semi- and non-inducible animals were relatively non-filicidal during the first week of PCPA treatment, and non-inducible animals were non-filicidal even at the end of three weeks of PCPA treatment. Since low brain serotonin does not invariably lead to filicide, one must assume that either other neurotransmitter systems and/or other behavioral factors may be instrumental in modulating the filicidal behavior. It is conceivable, however, that semi- and non-inducible animals represent the most docile animals of the population, or perhaps a hybrid sub-group more resistant to the PCPA regimen [19]. These behavioral differences may have been due to non-depleted levels of 5-HT in localized areas of the brain. If such differences did exist they would not have been detectable by measuring whole brain extract because of the dilution by portions of the brain normally low in 5-HT.

A plausible hypothesis could also be based on a relative decrease in the serotonergic system to that of a counterbalancing neurotransmitter activating system which mediates aggressive behavior. Indeed, some investigators have shown that PCPA may decrease other brain catecholamines which may be involved in mediating aggressive behavior [13,16]. Although reduced, the catecholamine content could be sufficiently elevated in reference to serotonin so that this system would become dominant. The relative functional relationship between these catecholaminergic and serotonergic systems may provide a clearer explanation for this type of aggressive behavior.

Clearly, once a female animal is potentiated, one or more sensory stimuli alerts and attracts the adult animal to the pup, and in turn, initiates an attack. Auditory and/or olfactory stimuli appear to be involved in the initial alerting and fixing process. It may be that the adult is reacting to cues that are perceived as offensive or threatening. Close proximity to the pup, per se, does not appear to be a major factor, as a filicidal animal will seek out a pup in an enjoining cage connected by a tunnel. We would conjecture that in this situation, auditory clues such as mewing or rustling sound produced by movement of the pup in the litter, may be the primary attractants [15].

It follows that once the object is located and fixed, continued auditory, together with visual (activity) and possible olfactory stimuli become important factors in the precipitation of an attack. However, olfactory cues may not be the primary eliciting factor, as prepartum animals whose nasal passages had been treated prior to parturition with liberal quantities of silicone grease, petroleum jelly, and petroleum jelly containing tentative olfactory masking preparations, also destroyed their offspring. Similarly, an attack can occur with little or no apparent evidence of visible activity on the part of a live pup. For example, nulliparious animals will also attack and 'kill' e.g., toy mice of different colors, an immobilized or dead pup, a live or dead pup enfolded in aluminum foil, a live mouse, or even a dead pup encased in solid plastic. Sex of the pup appeared to be of little importance as nulliparious animals were found to have little preference for male versus female pups.

It is possible that the stress associated with parturition is a minor factor in precipitating filicidal behavior in prepartum animals, however, the fact that both postpartum and nulliparious animals kill pups suggests that parturition is not a major causative factor. We would also discount the anorexia produced by PCPA as pre- and postpartum animals did not attack viable pups even when food deprivation produced excessive weight losses. We thus conclude that neither the parturition process nor severe food deprivation are strong causative factors in precipitating filicidal behavior.

At present, we view this inducible filicide phenomenon as being an expression of a natural latent aggressive tendency which is potentiated by the interference in, or reduction of, a suppressing system governed primarily by serotonin. Since it appears that not all animals are potentially filicidal, other biochemical and/or behavioral variables must also be involved.

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REFERENCES

1. Connor, R. L., J. M. Stolk, J. D. Barchas, W. C. Dement and S. Levine. The effect of *para*- chlorophenylalanine (PCPA) on shock-induced fighting behavior in rats. *Physiol. Behav.* 5: 1221–1224, 1970.
2. Connor, R. L., J. M. Stolk and S. Levine. Effects of PCPA on fighting behavior and habituation of startle response in rats. In: *Serotonin and Behavior*, edited by J. Barchas and E. Usdin. New York: Academic Press, 1973, pp. 322–333.
3. Copenhaver, J. H., M. J. Carver and R. L. Schalock. Experimental maternal hyperphenylalaninemia: biochemical effects and offspring development. *Devl. Psychobiol.* 7: 175–184, 1974.
4. Copenhaver, J. H., M. J. Carver, E. A. Johnson and M. J. Saxton. Effect of *para*-chlorophenylalanine on the growth and development of the fetal, neonatal, and adult rat. *Biochem. Med.* 4: 516–530, 1970.

5. DiChiara, G., R. Camba and P. F. Spano. Evidence for inhibition by brain serotonin of mouse-killing behavior in rats. *Nature* **233**: 272–273, 1971.
6. Domingues, M. and V. G. Longo. Taming effects of *para*-chlorophenylalanine on septal rats. *Physiol. Behav.* **4**: 1031–1033, 1969.
7. Ellison, G. D. and D. E. Bresler. Tests of emotional behavior in rats following depletion of norepinephrine, of serotonin, or both. *Psychopharmacologia* **34**: 225–278, 1974.
8. Gál, E. M. and S. A. Millard. The mechanism of inhibition of hydroxylases *in vivo* by *para*-chlorophenylalanine: the effect of cycloheximide. *Biochem. Biophys. Acta* **227**: 32–41, 1971.
9. Jequier, E., W. Lovenberg and A. Sjoerdsma. Tryptophan hydroxylase inhibition. The mechanism by which p-chlorophenylalanine depletes rat brain serotonin. *Mol. Pharmac.* **3**: 274–275, 1967.
10. Koe, B. K. and A. Weissman. p-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154**: 499–516, 1966.
11. Kulkarni, A. S., R. G. Rahwan and S. E. Bocknik. Muricidal block induced by 5-hydroxytryptophan in the rat. *Archs int. Pharmacodyn.* **201**: 308–313, 1973.
12. McLain, W. C. and B. T. Pole. Central catechol- and indoleamine systems and aggressions. *Pharmac. Biochem. Behav.* **2**: 123–126, 1977.
13. Miller, F., R. H. Cox, W. R. Snodgrass, Jr. and R. P. Maikel. Comparative effects of p-chlorophenylalanine, p-chloroamphetamine, and p-chloromethylamphetamine on rat brain norepinephrine, serotonin, and 5-hydroxyindole-3-acetic acid. *Biochem. Pharmac.* **21**: 435–442, 1970.
14. Moyer, K. E. Kinds of aggression and their physiological basis. *Comms behav. Biol.* **2** Part A: 65–67, 1968.
15. Noirot, E. Ultrasounds and maternal behavior in small rodents. *Devl Psychobiol.* **5**: 371–387, 1972.
16. Peters, D. A. V., M. Filczewski and I. M. Mazurkiewicz-Kwilecki. Effects of p-chlorophenylalanine upon catecholamine synthesis in rat brain, heart and adrenals. *Biochem. Pharmac.* **21**: 2282–2284, 1972.
17. Sheard, M. H. The effect of *para*-chlorophenylalanine on behavior in rats: relation to brain serotonin and 5-hydroxyacetic acid. *Brain Res.* **15**: 524–528, 1969.
18. Udenfriend, S. H., H. Weissbach and D. F. Bogdanski. Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan. *J. Biol. Chem.* **224**: 803–810, 1957.
19. Valzelli, L., E. Giocalone and S. Garattini. Pharmacological control of aggressive behavior in mice. *Eur. J. Pharmac.* **2**: 144–146, 1967.
20. Welch, A. S. and D. L. Welch. Effect of stress and p-chlorophenylalanine upon brain serotonin, 5-hydroxyindoleacetic acid and catecholamines in grouped and isolated mice. *Biochem. Pharmac.* **17**: 669–708, 1965.
21. Wise, C. D. An improved and simplified method for the fluorometric determination of brain serotonin. *Anal. Biochem.* **20**: 369–371, 1976.