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p-Chlorophenylalanine Alters Pacing of Copulation in Female Rats

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EMERY, D. E. AND R. L. MOSS. *p-Chlorophenylalanine alters pacing of copulation in female rats.* PHARMACOL BIOCHEM BEHAV 20(3) 337–341, 1984.—When tested in apparatus that allowed them to determine their contacts with conspecifics, including sexually active male rats, estrogen- and progesterone-treated female rats systemically treated with para-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, showed reduced frequency of coital contacts and reduced duration of interactions with sexually active males. PCPA administration did not alter contacts with noncopulating males or other females nor did treated females differ from controls in ability to display lordosis or posing, both stereotyped behaviors characteristic of female rats in behavioral estrus. It is suggested that the alteration of temporal pacing of copulation in the PCPA-treated females is a result of drug-induced changes in the processing of exteroceptive stimuli.

PCPA Copulatory pacing Reproductive behavior Feminine sexual behavior 5-Hydroxytryptamine

IN past research examining the neural mechanisms involved in feminine sexual behavior in the rat, the behavioral measure most commonly used has been the frequency of lordosis, a stereotyped posture shown in response to copulatory acts of the male rat. Female and male rats are usually tested for copulation in a small observation cage with no opportunity for the test animal to regulate its contacts with the stimulus animal/s.

Administration of para-chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase [18], has been reported to alter the frequency of lordotic behavior in the rat dependent upon the baseline copulatory ability of the animal. In female rats the baseline levels of copulation are usually manipulated by the dosages of hormones administered exogenously to ovariectomized subjects. The administration of low doses of estrogen alone to ovariectomized female rats produces lordotic behavior that is less than maximal, i.e., the female does not show lordosis to every copulatory act of the male. The administration of estrogen and progesterone typically produces maximal lordotic behavior and also the display of other stereotyped behaviors such as ear wiggling and posing or presenting directed toward the male. In ovariectomized females with low doses of estrogen treatment, the administration of PCPA has been reported to *increase* [10, 11, 25, 37], or leave unchanged [28, 30, 31], the frequency of lordotic behavior in a small testing cage situation, whereas administration of PCPA to ovariectomized females receiving estrogen plus progesterone has been reported to *decrease* the frequency of lordosis to masculine copulatory acts [10, 15, 16]. Clearly PCPA administration does not have simple facilitating effects upon lordotic behavior in the female rat as observed in the small testing cage.

Lordotic behavior, although crucial for receiving intravaginal stimulation from the male, is only one aspect of copulatory behavior in the female. Even in the small testing cage, females have been noted to display rejection and solicitation behaviors which may reflect underlying tendencies of the female to avoid and seek copulation, respectively [17].

When tested in a situation that allows the female more control over her interactions with sexually active males, estrogen- and progesterone-treated females do indeed alternate between seeking and actively avoiding copulation [3,26]. The temporal characteristics of the pacing of copulation in the female rat are dependent on the quantity and quality of the coital contacts received by the female. The copulatory acts of the male include mounts, intromissions and ejaculations. Mounts are not associated with vaginal stimulation of the females and the act of intromission involves less vaginal stimulation than that of ejaculation [4]. Females avoid engaging in coital contacts longer after receiving an ejaculation than after an intromission, and avoid coitus the least after mounts [4, 14, 21]. Thus the female rat by her pacing of copulation reflects a clear and precise sensitivity to the stimulus intensity or character of each coital contact.

It has been proposed that PCPA administration results in a hypersensitivity to supraliminal exteroceptive stimulation which is reflected by exaggerated behavioral responses to stimulation [35]. We reasoned that, if the stimulus characteristics of contacts with sexually active males appear enhanced or intensified following PCPA treatment, then, when tested in an apparatus giving her freedom to do so, the female should slow the pace of contacts with the males. The test situation used to examine this hypothesis allowed the test female to control her contacts with three different types

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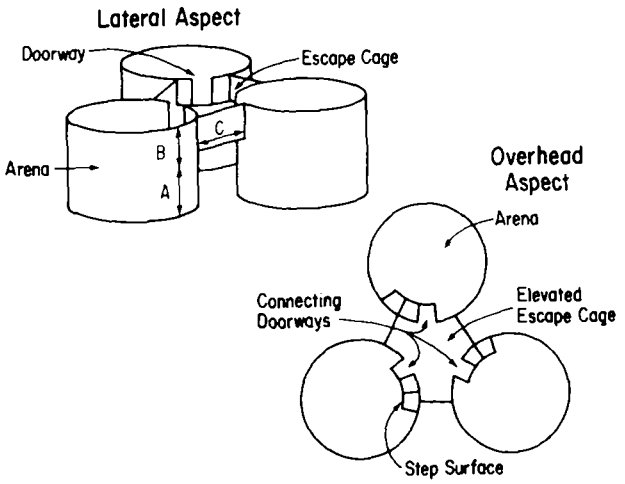

FIG. 1. Diagram views of the clear Plexiglas testing apparatus. Large circular arenas are 60 cm diameter and 45 cm high. Arenas are interconnected by an elevated ("A" = 25 cm from the floor of the arenas) triangular passageway (escape cage). The passageway is 20 cm high ("B"). The opening of the passageway to each arena is connected to the floors of the arenas by 3-step staircases (each step approximately 8.3×8.3×8.3 cm). The passageway has a removable clear lid, connecting sides between arenas (20 cm in length = "C") and floor surface of approximately 1150 cm^2. The steps have only a top and one lateral (front) surface and are connected on one side to the interior wall of their arena. The undersides of the steps are open, allowing the test females to crouch beneath the bottom step.

of stimulus animals: sexually active male rats, sexually inactive, but gonadally intact, male rats; and ovariectomized female rats. Each type of stimulus animal was contained in one of three separate large arenas interconnected by an elevated passageway (Fig. 1).

METHOD

Twenty-eight Long Evans (Charles River) female rats were ovariectomized at least three weeks before their predrug screening test. At approximately 150 days of age, the females received 20 μg/kg estradiol benzoate (EB), 48 hr before their predrug test, and 500 μg progesterone/animal, 4 hr before the test. One day before the test, the females were adapted for 45 min in the three-arena apparatus without stimulus animals present. The predrug and subsequent drug tests each consisted of a 10-min adaptation period in an empty three-arena apparatus and then a 10-min test in the apparatus with all the stimulus animals in place. One arena contained 3–5 sexually active males. When a male ejaculated or slowed in copulation he was replaced with a rested male. One arena contained three sexually passive males. The males were rendered passive by performing bilateral radiofrequency lesions in the medial preoptic area. The passive males investigated the test female but did not attempt to engage in copulatory acts. The last arena contained three ovariectomized (OVX) female rats with no hormone replacement; only the test female was allowed free entry to and egress from the arenas. The stimulus animals were discouraged from climbing the stairs by rapping them on the nose with a dowel rod.

During testing the following behaviors were scored by an observer: frequency and duration of visits by the test female to each arena; the copulatory acts of the active males and the accompanying lordotic behavior of the test female; and the stereotyped posture of posing in which the test female orients her hindquarters toward the stimulus animal. A derived measure of lordotic ratio was computed and is defined as (the frequency of lordotic postures associated with masculine copulatory acts divided by the total number of masculine copulatory acts) × 100. All behaviors and entries and egresses from the arenas were scored on an Esterline Angus event recorder.

During the predrug test the females had to spend over 10 sec in the active male arena to be continued in the study. Five females were discarded for failing to meet this criterion. Three days after the predrug test, 11 of the females began receiving the first of four daily injections of PCPA methyl ester hydrochloride, IP. PCPA methyl ester hydrochloride (100 mg/kg/day) was dissolved in 0.9% saline (100 mg/ml) and adjusted to a pH of 6.8–7.8. The remainder of the females (n=12) began receiving the first of four daily injections of the 0.9% saline (SAL) vehicle, IP. The dosage and schedule of administration of PCPA used in the present study produce profound depletion of 5-hydroxytryptamine and modest depletion of catecholamines [9]. On the third day of PCPA or vehicle treatment, all the females received 20 μg/kg EB and were tested in the three arena apparatus the day following their last PCPA or SAL treatment (EB-alone test). Immediately following the EB-alone test all females were injected with 500 μg progesterone and then retested 4 hr later (EB+progesterone test).

RESULTS AND DISCUSSION

During the predrug test the PCPA and SAL groups did not differ on any behavioral measure. All comparisons were made with the Student's t-test. In the EB-alone drug test the two groups differed on only one measure; the PCPA-treated females entered the active male arena less frequently than did the SAL females (Table 1). Two of the five PCPA-treated females that engaged in copulatory acts displayed lordotic behavior; one of the five SAL-treated females that copulated displayed lordosis. Both groups received equivalent amounts of copulatory contacts (mounts + intromissions + ejaculations).

During the EB+progesterone test, however, the two groups differed profoundly in the frequency of interactions with the sexually active males. Behavioral measures for the EB+progesterone test are presented in Table 2.The PCPA-treated females spent significantly less time and engaged in fewer copulatory acts with the active males than did the SAL-treated females. The groups differed not only in the total number of copulatory acts received but also in the frequency of contacts with presumed vaginal stimulation (intromissions and ejaculations). The PCPA-treated females received a mean of 3.1±1.67 intromissions and ejaculations which was significantly lower than the SAL females' mean of 8.6±2.03 ($p<0.02$). Despite the treatment effect on the amount of interaction with the active males, the groups did not differ in the total frequency of posing. PCPA-treated females displayed a mean of 13.3±4.44 poses whereas SAL-treated females displayed a mean of 14.2±4.0 poses.

Within group comparisons of the total durations spent in the passive male and OVX female arenas during the EB-alone test indicated that both the PCPA and SAL females spent more time in the OVX female arena, $t(10)=2.61$,

TABLE 1

THE EFFECTS OF PCPA ADMINISTRATION ON THE BEHAVIOR OF FEMALE RATS TREATED WITH ESTROGEN ALONE

	Measures							
	Total Time in Arenas with			Frequency of Entries to Arenas				
Group (N)	Active Males	Passive Males	OVX* Females	Active Males	Passive Males	OVX Females	Total M+I+E† Received	LR‡
PCPA (11)	25.5	98.4	330.5	1.9	1.6	7.0	2.0	6.0§
	±9.0	±44.13	±46.51	±0.44	±0.46	±0.95	±0.96	±4.0
	NS	NS	NS	$p<0.02$	NS	NS	NS	NS
Saline (12)	69.7	41.8	256.8	4.8	2.0	8.9	3.3	12.4§
	±23.45	±13.77	±29.86	±1.15	±0.39	±0.83	±1.93	±12.38

Data are expressed as group means ± standard errors.
Temporal measures are in seconds.
*OVX=ovariectomized.
†M=mount; I=intromission; E=ejaculation.
‡LR=lordotic ratio.
§N=5 for the group.

TABLE 2

THE EFFECTS OF PCPA ADMINISTRATION ON THE BEHAVIOR OF FEMALE RATS TREATED WITH ESTROGEN AND PROGESTERONE

	Measures							
	Total Time in Arenas with			Frequency of Entries to Arenas				
Group (N)	Active Males	Passive Males	OVX* Females	Active Males	Passive Males	OVX Females	Total M+I-E‡ Received	LR‡
PCPA (11)	16.7	150.2	284.6	1.9	1.8	6.5	4.5	83.3§
	±3.35	±52.23	±58.97	±0.39	±0.57	±1.25	±1.01	±11.41
	$p<0.007$	NS	NS	$p<0.005$	NS	NS	$p<0.002$	NS
Saline (12)	78.9	127.3	215.5	4.1	2.7	8.7	15.0	100.0
	±22.06	±38.45	±31.18	±0.61	±0.68	±1.29	±2.69	±0.0

Data are expressed as group means ± standard errors.
Temporal measures are in seconds.
*OVX=ovariectomized.
†M=mount; I=intromission; E=ejaculation.
‡LR=lordotic ratio.
§N=10 for the PCPA group.

$p<0.02$ and $t(11)=5.54$, $p<0.001$, respectively. The PCPA and SAL females also entered the OVX female arena more frequently in comparison to the passive male arena, $t(10)=4.48$, $p<0.001$ and $t(11)=6.39$, $p<0.001$, respectively. The preference shown for the OVX female arena by the females during the EB-alone test was not displayed during the EB+progesterone test since the within group comparisons were not significantly different for either group on the total durations spend in the passive male arena in comparison to the OVX female arena. The PCPA- and SAL-treated females did enter the OVX female arena more frequently than the passive male arena during the EB+progesterone test $t(10)=2.86$, $p<0.01$ and $t(11)=3.77$, $p<0.002$, respectively.

The PCPA-treated females did not appear debilitated or sluggish and the groups did not differ significantly on any other variable. The group differences in the lordotic ratio approached significance on the EB+progesterone test ($p<0.07$) but the low mean of the PCPA-treated females (Table 2) was due entirely to two females, both of whom spent less than 12 sec with the active males and received three or fewer copulatory acts in their single brief visit. One PCPA-treated female did not enter the active-male arena and the rest of the females had lordotic ratios of 100.

With estrogen treatment alone both groups of females in the present study were hesitant to engage in copulation, indicating that PCPA treatment did not increase the preferred frequency of coital contacts in estrogen-alone females. Previous reports of enhanced lordotic behavior following PCPA treatment to estrogen-treated females [10, 11, 25, 37] may have been due to the use of the small-cage-testing paradigm in which the females cannot avoid receiving mounts. If

PCPA treatment increases the preceived intensity of the mounting stimulation, then enhanced lordosis may be a result of PCPA treatment to females with a threshold level of estrogen. The lowered frequency of lordosis to mounting after PCPA administration reported in previous studies using estrogen- and progesterone-treated females [10, 15, 16] may have reflected the presently reported decrease in preferred frequency of copulation: the drug-treated females may have attempted to slow the pace of coital contacts by displaying avoidance behaviors incompatible with lordosis.

Systemic administration of PCPA enhances homosexual mounting behavior [29] and slightly enhances [27] or produces no effect [36] on the heterosexual copulation of sexually vigorous males, but produces dramatic enhancement of heterosexual copulation of sexually sluggish males [24]. PCPA administration also increases the display of masculine copulatory behavior in ovariectomized female rats [30]. To compare the present results with those found in masculine copulatory behavior following PCPA treatment, it is necessary to examine how exteroceptive stimuli modulate masculine copulation. Increasing the intensity of exteroceptive stimuli (both sexual and nonsexual) enhances masculine copulatory activity as reflected by changes in a number of behavioral measures, including a reduction in the intervals between intromissions [2, 6, 22, 23]. The same alterations in masculine copulation are produced by PCPA treatment [27]. Thus both feminine and masculine copulatory behaviors appear to be altered, following PCPA administration, as though the intensity of exteroceptive stimuli was increased.

PCPA treatment is also reported to modify other behaviors, including ingestive behavior, particularly to distinctively flavored substances [5, 8, 32], startle responses [7], acquisition of avoidance behavior [19,33], investigation of novel objects [13] and response to pain [12,33]. What appears to be consistent among these behaviors is the relative dependence of the behaviors on characteristics of the exteroceptive stimulation as opposed to fluctuations in internal stimuli. Of particular interest in relation to the present study, is the recent observation that the effects of PCPA administration on avoidance behaviors generally parallel the effects of increased shock intensity on avoidance conditioning [19]. Sensory thresholds are not necessarily changed by PCPA treatment [12,33] and PCPA effects are not limited to behavioral changes but are also found to alter neuroendocrine responses, particularly those responses that are sensitive to exteroceptive stimulation [20,34].

Since PCPA administration produces substantial depletion of 5-hydroxytryptamine (5-HT), the present results and the studies cited above implicate tryptaminergic systems in the transmission of information concerning characteristics of external stimuli that transcend specific sensory modalities and that influence diverse categories of behavior. However, the temporal characteristics of 5-HT depletion and behavioral changes following PCPA administration are not isomorphic indicating that alterations in non-tryptaminergic systems may contribute to the effects of PCPA treatment [1].

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REFERENCES

1. Ahlenius, S., J. Engel, H. Eriksson, K. Modigh and P. Södersten. Importance of central catecholamines in the mediation of lordosis behavior in ovariectomized rats treated with estrogen and inhibitors of monoamine systhesis. *J Neural Transm* **33:** 247–255, 1972.
2. Barfield, R. J. and B. D. Sachs. Sexual behavior: stimulation by painful electrical shock to skin in male rats. *Science* **161:** 392–393, 1968.
3. Bermant, G. Response latencies of female rats during sexual intercourse. *Science* **133:** 1771–1773, 1961.
4. Bermant, G. and W. H. Westbrook. Peripheral factors in the regulation of sexual contacts by female rats. *J Comp Physiol Psychol* **61:** 244–250, 1966.
5. Brody, J. F., Jr. Behavioral effects of serotonin depletion and of p-chlorophenylalanine (a serotonin depletor) in rats. *Psychopharmacologia* **17:** 14–33, 1970.
6. Caggiula, A. R. and M. Vlahoulis. Modifications in the copulatory performance of male rats produced by repeated peripheral stimulation. *Behav Biol* **11:** 269–274, 1974.
7. Conner, R. L., J. M. Stolk, J. D. Barchas and S. Levine. Parachlorophenylalanine and habituation to repetitive auditory startle stimuli in rats. *Physiol Behav* **5:** 1215–1219, 1970.
8. Ellison, G. D. and D. E. Bresler. Tests of emotional behavior in rats following depletion of norepinephrine, of serotonin, or of both. *Psychopharmacologia* **34:** 275–288, 1974.
9. Emery, D. E., J. Engel and K. Larsson. Rat strain differences in brain monoamine metabolism following para-chlorophenylalanine treatment. *Pharmacol Biochem Behav* **12:** 311–314, 1980.
10. Emery, D. E. and K. Larsson. Rat strain differences in copulatory behavior after para-chlorophenylalanine and hormone treatment. *J Comp Physiol Psychol* **93:** 1067–1084, 1979.
11. Everitt, B. J., K. Fuxe, T. Hökfelt and G. Jonsson. Role of monoamines in the control by hormones of sexual receptivity in the female rat. *J Comp Physiol Psychol* **89:** 556–572, 1975.
12. Fibiger, H. C., P. H. Mertz and B. A. Campbell. The effects of para-chlorophenylalanine on aversion thresholds and reactivity to foot shock. *Physiol Behav* **8:** 259–263, 1972.
13. File, S. E. Effects of parachlorophenylalanine and amphetamine on habutuation of exploration. *Pharmacol Biochem Behav* **6:** 151–156, 1977.
14. Gilman, D. P. and J. C. Hitt. Effects of gonadal hormones on pacing of sexual contacts by female rats. *Behav Biol* **24:** 77–87, 1978.
15. Gorzalka, B. B. and R. E. Whalen. Inhibition not facilitation of sexual behavior by PCPA. *Pharmacol Biochem Behav* **3:** 511–513, 1975.
16. Hamburger-Bar, R., H. Rigter and I. Dekker. Inhibition of serotonin reuptake differentially affects heterosexual behavior of male and female rats. *Life Sci* **22:** 1827–1836, 1978.
17. Hardy, D. F. and J. F. DeBold. Effects of coital stimulation upon behavior of the female rat. *J Comp Physiol Psychol* **78:** 400–408, 1972.
18. Koe, B. K. and A. Weissman. p-Chlorophenylalanine: A specific depletor of brain serotonin. *Pharmacol Exp Ther* **154:** 499–516, 1966.
19. Köhler, C. and S. A. Lorens. Open field activity and avoidance behavior following serotonin depletion: A comparison of the effects of para-chlorophenylalanine and electrolytic midbrain raphe lesions. *Pharmacol Biochem Behav* **8:** 223–233, 1978.
20. Kordon, C. and V. D. Ramirez. Recent developments in neurotransmitter-hormone interactions. In: *Anatomical Neuroendocrinology*, edited by W. E. Stumpf and L. D. Grant. New York: S. Karger, 1975, pp. 409–419.

21. Krieger, M. S., D. Orr and T. Perper. Temporal patterning of sexual behavior in the female rat. *Behav Biol* **18:** 379–386, 1976.
22. Larsson, K. Non-specific stimulation and sexual behaviour in the male rat. *Behaviour* **20:** 110–114, 1963.
23. Madlafousek, J., Z. Hliňák and J. Beran. Decline of sexual behavior in castrated male rats: Effects of female precopulatory behavior. *Horm Behav* **7:** 245–252, 1976.
24. Malmnäs, C. O. and B. J. Meyerson. P-Chlorophenylalanine and copulatory behaviour in the male rat. *Nature* **232:** 398–400, 1971.
25. Meyerson, B. J. and T. Lewander. Serotonin synthesis inhibition and estrous behavior in female rats. *Life Sci* **9:** 661–671, 1970.
26. Peirce, J. and L. Nuttall. Self-paced sexual behavior in the female rat. *J Comp Physiol Psychol* **54:** 310–313, 1961.
27. Salis, P. J. an D. A. Dewsbury. The effects of p-chlorophenylalanine on copulatory behavior of male rats. *Nature* **232:** 400–401, 1971.
28. Segal, D. S. and R. E. Whalen. Effect of chronic administration of p-chlorophenylalanine on sexual receptivity of the female rat. *Psychopharmacologia* **16:** 434–438, 1970.
29. Sheard, M. The effect of p-chlorophenylalanine on behavior in rats: relation to brain serotonin and 5-hydroxyindolacetic acid. *Brain Res* **15:** 524–528, 1969.
30. Singer, J. J. Effects of p-chlorophenylalanine on the male and female sexual behavior of female rats. *Psychol Rep* **30:** 891–893, 1972.
31. Södersten, P., K. Larsson, S. Ahlenuis and J. Engel. Stimulation of mounting behavior in ovariectomized female rats by p-chlorophenylalanine. *Pharmacol Biochem Behav* **5:** 329–333, 1976.
32. Stein, J. M., M. J. Wayner and H. A. Tilson. The effect of para-chlorophenylalanine on the intake of ethanol and saccharin solutions. *Pharmacol Biochem Behav* **6:** 117–122, 1977.
33. Tenen, S. S. The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
34. Vernikos-Danellis, J., P. Berger and J. D. Barchas. Brain serotonin and pituitary-adrenal function. In: *Progress in Brain Research*, vol 39, edited by E. Zimmerman, W. H. Gispen, B. H. Marks and D. deWied. Amsterdam: Elsevier, 1973, pp. 301–309.
35. Weissman, A. and C. A. Harbert. Recent developments relating serotonin and behavior. *Ann Rep Med Chem* **7:** 47–58, 1972.
36. Whalen, R. E. and W. G. Luttge. p-Chlorophenylalanine methyl ester: An aphrodisiac? *Science* **169:** 1000–1001, 1970.
37. Zemlan, F. P., I. L. Ward, W. R. Crowley and D. L. Margules. Activation of lordotic responding in female rats by suppression of serotonergic activity. *Science* **179:** 1010–1011, 1973.