Pharmacology Biochemistry & Behavior, Vol. 11, pp. 701-704. Printed in the U.S.A.

PCPA Potentiates the Effects of Specific Copulatory Experience on the Sexual Behavior of the Pudendectomized Male Rat

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Received 27 August 1979

DAHLÖF, L.-G. AND K. LARSSON. *PCPA potentiates the effects of specific copulatory experience on the sexual behavior of the pudendectomized male rat.* PHARMAC. BIOCHEM. BEHAV. 11(6) 701-704, 1979.—The interactional effects of bilateral pudendectomy and sexual experience were studied on the sexual behavior of male rats. In Experiment 1, limited sexual experience in terms of mounts, intromissions or ejaculations was provided prior to the operation. The sexual behavior was then tested before and after treatment with PCPA, a drug known to increase the sexual activity of male rats. In Experiment 2, massive sexual experience was provided the animals prior to pudendectomy, and the sexual behavior was tested with or without PCPA treatment. No variety of limited sexual experience differentiated the effects of pudendectomy. After PCPA treatment, however, the intromittive and ejaculatory capacity of all experienced animals improved significantly. In animals with massive sexual experience the behavioral dysfunctions after pudendectomy were even more dramatically counteracted by PCPA treatment.

Male rat PCPA Pudendal nerve section Sexual behavior Sexual experience

IMPAIRMENT of the sensory inflow from the penis by local anesthetization of the glans [1,2], or transection of the dorsal penile nerve [3, 4, 5, 6] causes serious deficits in the sexual behavior of the rat. The operated animal shows difficulty in achieving intromission and ejaculation displaying an increased proportion of mounts without intromission. Massive heterosexual experience prior to the operation partly compensates for the sexual deficits—an effect which may be explained as a conditioning to non-genital stimuli and/or a disinhibition of stimuli interfering with sexual activity.

Treatment of male and female rats with para-chlorophenylalanine (PCPA), a drug that selectively inhibits the synthesis of 5-hydroxytryptamine (5-HT), facilitates the display of mounting behavior [7, 9, 11]. Even rats rendered sexually inactive by castration may exhibit complete masculine mating pattern after treatment with PCPA. The mechanism by which PCPA produces these behavioral effects can only be speculated upon. Perhaps PCPA, by depressing the brain 5-HT levels, releases a neural mechanism mediating the mating pattern from a tonic inhibition thereby facilitating the expression of sexual behavior. Assuming that this PCPA-induced disinhibition increases the sensitivity to sexual stimulation, treatment of penile deafferentated males with PCPA may counteract the deficits caused by loss of penile sensations. This effect should be more marked in experienced rats than in inexperienced ones, because experienced rats have acquired the capacity of responding sexually to a wide range of non-genital stimuli.

Two experiments were carried out to explore this hypothesis. In a first experiment, early isolated male rats were allowed to acquire a limited amount of specified sexual experience as mounts, intromissions, or ejaculations prior to pudendectomy. They were thereafter tested for sexual behavior before and after PCPA treatment. In a second experiment, the animals were reared under normal conditions and provided massive heterosexual experience before experimentation.

GENERAL METHOD

Animals

Animals were male Wistar rats bred in the laboratory. They were maintained under artifically reversed dark-light conditions (dark 12:00–22:00). Humidity (50%) and temperature (22°C) were controlled automatically. Food and water were available ad lib. All animals were housed in standard cages 50×30 cm.

Surgery

The details of bilateral pudendal nerve transection are described elsewhere [3].

PCPA Treatment

Para-chlorophenylalanine (PCPA, 126 mg/kg, IP) was administered for 3 consecutive days and testing for sexual behavior was made on the 4th day. Control treatment (0.5 ml/rat isotonic saline) had the same schedule.

Necropsy

Three animals selected randomly from each group were perfused under pentobarbital sodium anesthesia by saline and 10% formalin. The penis was cut just distal to the bulbocavernous muscle fixed in 10% formalin, decalcified, and embedded in paraffin. The sections (8 μm) were impregnated according to the method for selective silver staining of nerve fibers [8]. No histological evidence of regeneration of the dorsal penile nerve could be demonstrated.

Mating Behavior

Mating tests were conducted in a circular Plexiglas arena (60 cm in dia.); the floor was covered with wood shavings. Depending upon the performance of the male, testing was ended after (1) a maximum of 15 min when no intromission was performed, or (2) a maximum of 30 min when at least one intromission was performed.

The following behavior measures were recorded: (a) mount: mount with pelvic thrusting; (b) intromission: mount with vaginal penetration; (c) ejaculation; (d) contact latency: interval between the presentation of the female and the first mount or intromission; (e) ejaculatory latency: period between the first intromission and ejaculation; (f) postejaculatory interval: interval between ejaculation and the first intromission after ejaculation.

The following derived measures were also calculated: (g) intromissions per minute: number of intromissions divided by the time from the first mount or intromission to ejaculation or when no ejaculation took place; to the time the test was finished according to the criteria stated above; (h) total sexual activity per minute: the number of mounts and intromission divided by same time measure in (g); (i) copulatory efficiency (%):

$$\frac{\text{number of intromissions} \times 100}{\text{number of mounts} + \text{intromissions}}$$

Procedure

Experiment 1. From the litters born at a given day, male pups were collected and randomly assigned to new litters, consisting of eight male pups. To minimize the influence of visual stimulation and climbing experience the mother was removed from her litter at Day 14, and the animals were fed a special protein-rich milk substitute manufactured by Semper AB. Fifteen milliliters of warmed food (35°C) was served in small petri dishes twice a day. Ordinary rat food and water were available ad lib. Cotton was placed in the cage as nesting material. At Day 25, the replacement feeding procedure was terminated, and the nesting material was withdrawn.

When 75 days old the animals were randomly assigned to any of the following four groups: Group IS (isolated, n=13), the animals remained isolated throughout the experiment except for the final testing occasions. Group M (mount, n=13), the animals were allowed to mount an estrous female rat during 15 min. Intromission is prevented by a rectangular piece of cotton cloth (15×30 mm) that covers the genital region of the female, and held in place by tying the strings over the back (Fig. 1). Group MI (mount+intromission, n=13), the animals allowed to mount and intromit an estrous female 10 times within 15 min. Group MIE (mount+intromission+ejaculation, n=12), the animals were allowed to proceed until ejaculation occurred. If the animal had any intromissions but did not ejaculate within 15 min, the test was prolonged with 15 min. Each group had 10 training sessions, one per day.

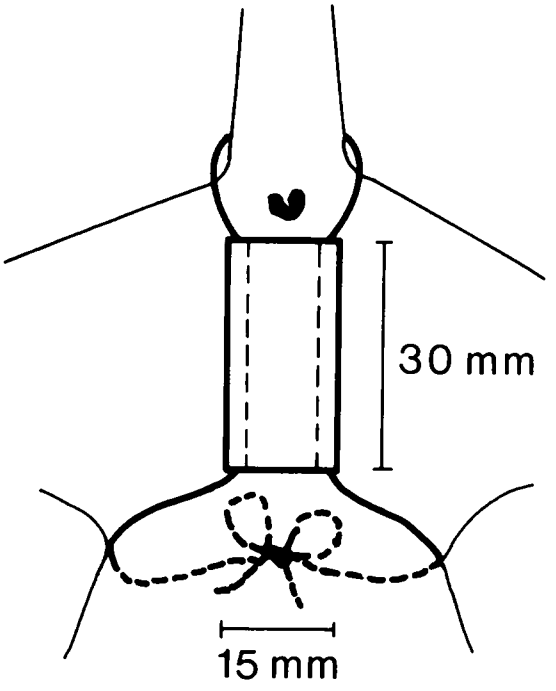

FIG. 1. Device for preventing vaginal penetration. Ventral view.

At 90 days of age bilateral pudendectomy was performed and after a 5-day recovery interval the animals were tested for sexual behavior. Another behavior test was made at 100 days of age after PCPA treatment.

Experiment 2. Animals were weaned at 25 days of age and then housed in groups of four males per cage. Between 70–90 days of age two males lived together with two receptive females. The females were brought into sexual receptivity by injection of estradiol benzoate (60 μg/rat) followed after 24, 48 and 72 hr by progesterone (0.5 mg/rat). After this 3-day regimen the females were replaced by new females treated similarly. At 90 days of age the animals were randomly assigned to any of the following groups: Intact-Control (IC, n=12); Intact-PCPA (IP, n=12); Operated-Control (OC, n=12) and Operated-PCPA (OP, n=12). The four groups were subjected to any combination of sham-operation, pudendectomy, saline or PCPA treatment and tested for sexual behavior at 95 days of age.

RESULTS

Experiment 1

Table 1 shows the results of this experiment. Before the drug treatment, no group differences were found in any behavioral measure except for the contact latency which was shorter in the three experienced groups. Following the drug treatment, the contact latency of the isolated rats was shortened to the level of the experienced rats. Further, the experienced rats showed a higher number of intromissions per minute and a higher copulatory efficiency percentage than did the isolated rats. Comparing the behavior before and after the drug treatment in each of the experienced

TABLE 1

MEASURES OF THE MATING BEHAVIOR DISPLAYED IN *EXPERIMENT 1*. (FISHER EXACT PROBABILITY TEST, KRUSKAL-WALLIS ONE-WAY ANALYSIS OF VARIANCE, MANN-WHITNEY U-TEST, FRIEDMAN TWO-WAY ANALYSIS OF VARIANCE AND WILCOXON MATCHED-PAINS SIGNED-RANKS TEXT) [10]

Parameter		Isolated (IS)	after PCPA	Mount (M)	after PCPA	Mount+Introm. (MI)	after PCPA	Mount+Introm.+Ejac. (MIE)	after PCPA
Number of animals		13	13	13	13	13	13	12	12
Number of animals displaying:									
Mounts		11	11	9	8	12	10	11	9
Intromissions		6	3	6	5	10	8	8	6
Ejaculations		1	3	0	5[a]	1	8[b]	1	6[c]
Ejaculation latency (min)	Md	13.55	15.00	—	19.80	15.25	7.18	26.20	14.23
Postejaculatory interval (min)	Md	8.50	6.45	—	6.50	9.70	5.83	6.85	6.70
Contact latency (min/100)	Md	110[k]	10	25	13	20	8	10	20
Intromission/min	Md	0.00	0.00[l]	0.00	0.38[d]	0.00	0.42[e]	0.00	0.17[f]
Total activity/min	Md	1.41	2.70[g]	1.34	1.36	1.87	2.21	1.44	1.93
Copulatory efficiency (%)	Md	0.00	0.00[e]	0.00	20.5 [h]	0.00	17.4 [i]	0.00	7.7 [j]

Comparisons within groups: [a,b]$p<0.02$; [c]$p<0.05$; [d,f]$p<0.05$; [e]$p<0.02$;[g,h,j]$p<0.05$; [i]$p<0.02$.
Comparisons between groups: [k]IS>MI=MIE $p<0.05$; [l]IS<M=MI=MIE $p<0.05$.

groups, the number of intromissions per minute and the copulatory efficiency percentage showed an increase in all groups. Between the various groups of experienced animals, no differences were found in any behavioral measure. In the isolated group only the total activity per minute increased as a result of the drug treatment. The number of ejaculating animals increased significantly after PCPA treatment in all experienced groups.

Experiment 2

The number of ejaculating animals in the Operated-Control group was significantly smaller compared to the other groups (Table 2). In the Operated-PCPA group this number was lower than in both intact groups. Comparing the PCPA treatment effect on the various behavior measures, the ejaculatory latency and the postejaculatory interval were significantly shorter in the Intact-PCPA group than in the Intact-Control group.

The Operated-PCPA rats showed a significantly higher total sexual activity than the Intact-Control animals. This may appear surprising but is most likely a joint effect of pudendectomy causing an increased number of mounts and a PCPA-induced increase of the intromission frequency. Finally, the pudendectomized rats showed a dramatically reduced copulatory efficiency percentage. However, this effect was to a large extent compensated for by the PCPA treatment.

DISCUSSION

Penile deafferentation seriously interfered with the mating performance of the sexually experienced rats in Experiment 2. The consequences of the operation were even more disastrous to the naive rats in Experiment 1. Although the animals were sexually motivated as was demonstrated by their pursuance and repeated mounting of the female, only a few of them intromitted and even fewer ejaculated.

The main finding of this study is that treatment with PCPA enhances the sexual activity of sexually experienced males having no or little influence on the sexual behavior in inexperienced rats. The effect of the drug treatment varied with the amount of experience provided the animal. Experience of specific components of the mating pattern during a few preoperative tests was less effective in facilitating the expression of sexual behavior than massive heterosexual experience acquired during several days of housing with receptive females. Interestingly, performing mounts, intromissions or ejaculations induced no effects recognizable under normal mating conditions. It may be speculated that during mating, the male learns to respond to a wide range of stimuli by sexual behavior. Sometimes such stimuli may be too weak to provoke any sexual behavior under normal mating conditions. By increasing the responsiveness of the animal to any stimulation, PCPA renders even weak sexual stimuli capable of provoking sexual activity. The very fact that PCPA did not influence the sexual behavior of the naive animals emphasizes that PCPA should not be considered as a stimulus to sexual activity but instead as a means to facilitate the expression of sexual behavior once adequate stimuli are present.

Observing that heterosexual experience was superior to homosexual experience in counteracting the impairing influence of penile deafferentation [4], we believed that the various components of the heterosexual copulatory pattern were specifically related to this effect. Present results showing no differences in the effects of the varieties of sexual experience provided by mounts, intromissions and ejaculations do not lend support to this assumption. It rather ap-

TABLE 2

MEASURES OF THE MATING BEHAVIOR DISPLAYED IN *EXPERIMENT II*. (FISHER EXACT PROBABILITY TEST, KRUSKAL-WALIS ONE-WAY ANALYSIS OF VARIANCE AND MANN-WHITNEY U-TEST)

GROUPS Parameter	Intact–Control (IC)	Intact–PCPA (IP)	Operated–Control (OC)	Operated–PCPA (OP)
Number of Animals	12	12	12	12
Number of animals displaying:				
Mounts	12	12	12	12
Intromissions	12	12	8	11
Ejaculations	12	12	1[a]	7[a]
Ejaculatory latency (min)	Md 10.45[b]	Md 3.03[b]	Md 9.05	Md 6.60[b]
Postejaculatory interval (min)	6.70[c]	4.70[c]	6.15	4.50
Contact latency (min/100)	13	10	10	10
Intromissions/min	0.77[d]	1.71[d]	0.03[d]	0.63[d]
Total activity/min	1.01[e]	2.14[e]	1.63[e]	3.47[e]
Copulatory efficiency (%)	42.5[f]	100.0[f]	1.7[f]	20.8[f]

[a]OC<OP<IP=IC $p<0.05$; [b]IP<OP=IC $p<0.02$; [c]IP<IC $p<0.02$; [d]IP>IC=OP>OC $p<0.01$; [e]IC<IP=OC=OP $p<0.02$; [f]IP>IC>OP>OC $p<0.05$ $p<0.02$ $p<0.002$.

pears that heterosexual activity instead of providing the male with experience of specific motor skills, promotes social interaction in which sexual behavior can be carried out.

Present findings do not support the claim that a functional pudendal nerve is a prerequisite for intromission [6]. Since the erectile and ejaculatory reflexes can be elicited after pudendectomy, the afferent neural activity necessary to produce these reflexes can not or only partly be mediated by the pudendal nerve. On the afferent side, the presence of both these reflexes in the pudendectomized rat suggests that the proximal part of the penis contains mechanoreceptors sending fibers through other nerves than the pudendal nerve.

To conclude, confirming previous findings that sexual experience acquired prior the operation, diminish the impairing influence of penile deafferentation, present results, in addition show that such effects, even if they not easily can be demonstrated under normal mating conditions, still may exist influencing the behavior and can be revealed under adequate conditions of observation.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Swedish Social Research Council. PCPA was generously supplied by Hässle AB, Mölndal, and the milk substitute was kindly provided by Semper AB, Stockholm. Håkan Lindgren and Marian Adamski are thanked for technical assistance, expert testing and devoted care of the animals.

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