Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 81 (2005) 871 – 878

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Prenatal blockade of androgen receptors reduces the number of intromissions needed to induce conditioned place preference after paced mating in female rats

Emilio Domínguez-Salazar, Francisco J. Camacho, Raúl G. Paredes *

Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, Qro. 76230, México

Received 17 December 2004; received in revised form 8 June 2005; accepted 14 June 2005
Available online 11 July 2005

Abstract

Estrous female rats pace their coital contacts to control the rate of cervical/vaginal stimulation. Females that receive at least 10 paced intromissions develop a reward state, evaluated by conditioned place preference (CPP), whereas females that do not pace their coital contacts do not develop CPP. We asked if the blockade of androgen receptors could modify the number of intromissions needed during paced mating to develop CPP. Pregnant females received daily injections of the androgen receptor antagonist flutamide from day 12 of pregnancy until pups were born. When adults, females exposed prenatally to flutamide or vehicle were ovariectomized and hormonally primed to evaluate if paced and non-paced mating induced CPP. The prenatal flutamide treatment did not affect the capacity of females to develop CPP to a systemic morphine injection. Flutamide-treated females that paced their sexual contacts developed CPP with fewer intromissions than control females. No effect on conditioning was observed when females were not allowed to pace their sexual contacts. The results suggest the existence of a threshold of cervical/vaginal stimulation that correlates with the induction of a reward state and that this threshold can be reduced by prenatal blockade of androgen receptors.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Conditioned place preference; Paced mating; Female rats; Flutamide

1. Introduction

Sexual behavior in female rats has a receptive and a proceptive component. Receptivity is characterized by the lordosis posture: the female rat arches her back, raises her head and rear, and deflects her tail, allowing the male rat to intromit (Beach, 1976). Proceptivity is a complex pattern of soliciting behaviors (hopping, darting, ear-wiggling, soliciting mounting), that triggers copulatory mounts from the male (Beach, 1976; Madlafousek and Hlinak, 1977; Blaustein and Erskine, 2002). Under semi-natural conditions (McClintock and Adler, 1978), in the wild (Calhoun, 1962), or in laboratory settings (Erskine and Baum, 1982a; Fang et al., 2000; Gans and Erskine, 2003; Lee and Erskine, 2000; Pfaus et al., 1999; Yang and Clements, 2000), estrous female rats exhibit a recognizable solicitation pattern consisting of approach toward, orientation to, and withdrawals from the male (McClintock and Adler, 1978). This pattern, named paced mating, has behavioral and physiological characteristics that are crucial for female sexual reproduction (reviewed in Blaustein and Erskine, 2002; Erskine, 1989). We have shown that paced mating behavior in females induces a reward state, evaluated by conditioned place preference (CPP), whereas non-paced mating does not induce CPP (Martinez and Paredes, 2001; Paredes and Alonso, 1997; García-Horsman and Paredes, 2004). In males, sexual behavior also induces a reward state (Hughes et al., 1990; Mehrara and Baum, 1990; Miller and Baum, 1987), and ejaculation appears to be an important factor for

* Corresponding author. Instituto de Neurobiología, Apartado Postal 1-1141, Querétaro, Qro. 76001, México. Fax: +52 442 234 0344.
E-mail address: rparedes@servidor.unam.mx (R.G. Paredes).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.06.011

the induction of CPP (Agmo and Berenfeld, 1990). In females, at least 10 paced intromissions, without receiving an ejaculation, are enough to reach an affective-positive state and induce conditioning (Paredes and Vazquez, 1999).

Male rats with prenatal androgen blockade, when adults, show a decrease in their masculine coital behavior (Clemens et al., 1978; Dominguez-Salazar et al., 2002) and an increase in lordosis behavior (Gladue and Clemens, 1978). Clemens et al. (1978) showed that androgenic substances secreted by the fetal testis of male rats influence the morphology (increase the ano-genital distance) and later behavior of nearby fetal females (increase male-like behavior). The changes in morphology and behavior observed in female rats located near a male during uterine development were blocked with the non-steroidal androgen receptor blocker flutamide (Clemens et al., 1978). However, treatment with the same androgen receptor antagonist had no effect on feminine coital behavior (Brand and Slob, 1991).

Females of others species, like hamster and ferrets, do not show male-like coital behavior such as that seen in rats. Female hamsters are not exposed prenatally to male-like levels of testosterone (Vomachka and Lisk, 1986), and in adulthood, they have a limited capacity to display male-typical coital behavior (Fiber and Swann, 1996; Noble, 1977). The transplacental administration of testosterone to female ferrets from embryonic days 16 to 34 (Baum, 1976) or from days 30 to 41, of a 41-day gestation period (Tobet and Baum, 1987), failed to augment their ability to exhibit masculine coital behavior, when tested in adulthood following ovariectomy and concurrent administration of testosterone. On the other hand, female ferrets do not pace their coital interaction (Baum, 1990). This information suggests to us that androgens, which induce male-like coital behavior and cross the transplacental barrier in female rats more easily than in female ferrets and hamsters, could probably reduce the rewarding properties of mating. If this is the case, pacing behavior could function to reduce these negative aspects. To test the hypothesis that the rewarding components of female rat sexual behavior could be modulated by androgenic influences in perinatal life, we evaluated the capacity of female rats with prenatal blockade of androgen receptors to develop CPP by paced and non-paced mating. In order to determine if the prenatal treatment modified the ability of the females to develop CPP, in experiment 1, we tested if females treated prenatally with flutamide develop CPP to a morphine injection. In the second experiment, we evaluated if flutamide treatment could reduce the number of intromissions needed during paced mating to develop CPP. Since, in previous studies, we have shown that at least 10 paced intromissions are required to induce CPP (Paredes and Vazquez, 1999), we allowed the females to receive no more than 9 intromissions. In the last experiment, we evaluated if prenatal flutamide treatment could induce CPP when females are not allowed to pace their sexual interaction.

2. General methods

2.1. Subjects

Sprague–Dawley rats obtained from the breeding colony at the Instituto de Neurobiología (INB, UNAM, Querétaro, México) were maintained in a room with a reversed light–dark cycle (12-h light/12-h dark; lights off at 9 AM) with food and water available ad libitum. The INB/UNAM Animal Care and Use Committee approved all procedures. Different animals were used for each experiment, and all subjects were sexually naive before the experiment.

2.2. Prenatal treatment

Nulliparous female rats were time-mated. Starting at day 12 of gestation, pregnant dams of the control group were injected with vehicle (VEH) twice daily until birth. Experimental dams were injected twice daily (10 AM and 8 PM) with flutamide (10 mg/kg, dissolved in propylene glycol with 10% ethanol) until pups were born. Since flutamide, and its active metabolite hydroxy-flutamide, have a short serum half-life (about 8 h; Fuh and Stoner, 1998), we injected the compound twice a day to increase the probability of blocking androgen receptors completely. This treatment has been successfully used in the past to block androgen receptors prenatally (Brand and Slob, 1991; Dominguez-Salazar et al., 2002). Moreover, male siblings of the flutamide-treated females had an ano-genital distance significantly reduced (3.6 cm) compared to male siblings from vehicle-treated females (5.27 cm). These observations clearly show that in the present experiment, flutamide treatment reached the pups and had effects similar to those previously described (Gladue and Clemens, 1978; Imperato-McGinley et al., 1992). Pups were weaned at 25 days of age and housed in groups of three to four of the same treatment and sex to a cage. When adults of approximately 3 months, females were gonadectomized under sterile conditions using ketamine (70 mg/kg) and xylazine (6 mg/kg).

2.3. Behavioral procedures

2.3.1. Place preference paradigm

Three weeks after surgery, females were tested in a three-compartment box. The central compartment ($22 \times 24 \times 32$ cm), painted gray, communicates with the lateral compartments ($23 \times 37 \times 32$ cm) through a sliding door (10×10 cm). The lateral compartments offer distinct stimuli in color, texture and odor. One compartment is white with sawdust on the floor, and the other is black with the walls moistened with a 2% solution of glacial acetic acid. The animals were observed through the front wall of the central compartment made of fine wire mesh. After determining the initial preference, females were primed with a hormone replacement treatment that induces high levels of proceptive and receptive behavior and has been used repeatedly in experi-

ments of CPP and mating (Martinez and Paredes, 2001; Paredes and Alonso, 1997; García-Horsman and Paredes, 2004). Estradiol benzoate (25 μg) and progesterone (0.5 mg) were injected 52 and 4 h, respectively, before exposing the females to the reinforcing event (morphine in experiment 1, mating in experiments 2 and 3). A procedure similar to that used by Paredes and Alonso (1997) was followed. Placing the subject in the middle compartment and recording the time spent in each of the lateral chambers during a 10-min session determined the initial preference (pretest). During conditioning sessions, the animals were placed in the preferred compartment for 30 min. On alternate days, the females were exposed to the reinforcing event and placed in the non-preferred (rewarded) compartment for 30 min. After six conditioning sessions, three reinforced and three non-reinforced, the preference for each chamber was tested again (test) in exactly the same way as before conditioning (pretest).

2.3.2. Paced and non-paced mating

The mating cages ($40 \times 60 \times 40$ cm) were equally divided by a removable wood partition with a small hole (4×7 cm) through which the female could enter or exit the other half of the cage in which the male was confined. In experiment 2, females paced their coital interaction; in experiment 3, the partition was removed and the females were not able to pace their sexual contacts. During mating tests, the following parameters of sexual behavior were recorded: latencies and number of mounts, intromissions and ejaculations. The interintromission interval (III, ejaculation latency divided by the number of intromissions) was calculated. Lordosis intensity was rated on a scale of 0–2 as follows: 0, no lordosis; 1, partial lordosis characterized by lateral tail deviation with moderate concave back flexion and neck extension; 2, full lordosis characterized by lateral tail deviation with pronounced back and neck flexion. In this way, the mean lordosis intensity (MLI, the sum of points divided by the number of mount plus intromission received) and the lordosis quotient (LQ, the total number of lordosis responses divided by the number of mounts and intromissions multiplied by 100) were calculated. For pacing behavior, percent of exits (percent of exits from the males' side of the pacing chamber following mounts or following intromissions or following ejaculation) and return latencies (time between an exit and the return to the cage in which the male was confined) after mount, intromission and ejaculation were calculated.

Table 1 summarizes the groups and treatments during conditioning for each experiment.

2.4. Statistical analysis

2.4.1. Sexual behavior

The number and latencies of mount, intromission, and ejaculation; the LQ and percent of exists after mount, intromission, or ejaculation were evaluated by an ANOVA for independent groups followed by LSD's Fisher post hoc test when the ANOVA revealed a significant effect. The return latencies after mount, intromission and ejaculation were evaluated by a 3 (group) × 3 (behavioral measure) ANOVA in order to compare within and between groups. In cases with a significant effect, an LSD's Fisher post hoc test was performed. For these parameters, we calculated the mean for each animal in each test and obtained the average of the three conditioning sessions.

2.4.2. Conditioned place preference

We used two criteria to consider that a treatment induced a change in place preference: the time in the reinforced compartment and the preference score (time in reinforced compartment/[time in reinforced compartment+time in non-reinforced compartment]). Both should increase between pretest and test. It was considered

Table 1
Prenatal treatments and reinforced events of each group in the three experiments reported

Experiment	Group name	*N*	Prenatal treatment of group	Treatment before being placed in the preferred compartment	Treatment before being placed in the reinforced compartment
1) Morphine	1) VEH-SAL	9	Vehicle	Saline	Saline
	2) VEH-MOR	9	Vehicle	Saline	Morphine
	3) FLU-MOR	10	Flutamide	Saline	Morphine
2) Paced mating	1) VEH-0-Intro	10	Vehicle	–	Without mating (0 intromission)
	2) VEH>10-Intro	10	Vehicle	–	More than 10 paced intromissions
	3) VEH<9-Intro	10	Vehicle	–	Less than 9 paced intromissions
	4) FLU<9-Intro	10	Flutamide	–	Less than 9 paced intromissions
3) Non-paced mating	1) VEH	10	Vehicle	–	More than 10 non-paced intromissions
	2) FLU	10	Flutamide	–	More than 10 non-paced intromissions

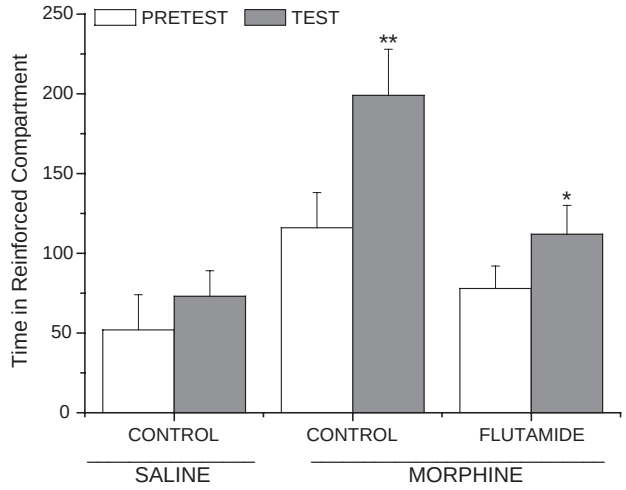

Fig. 1. Effect of morphine on CPP. Time (seconds) spent in the reinforced compartment in control and flutamide-treated females injected with morphine. Mean±S.E.M, *Different from pretest condition, $p<0.05$; **$p<0.01$.

important to use both criteria because either of them alone could indicate a false preference change. The preference score and the time spent in the reinforced compartment were evaluated by *t* tests. Since subjects were randomly assigned depending on the treatment, to the different groups before the pretest, the important comparison is within groups, that is, pretest vs. test. In this way, each animal serves as its own control. Comparisons between groups are not adequate because they could differ in their base line scores.

3. Experiment 1: conditioning with morphine

3.1. Methods

This experiment was designed to determine if the general reward system was modified by the prenatal flutamide treatment. It was important to show that a common manipulation that reliably induces CPP, a systemic injection of morphine, was also effective in our prenatally treated females. For this purpose, 18 vehicle-treated females and 10 flutamide-treated females were used. Females were injected with saline in the non-reinforced sessions. During reinforced sessions, of the 18 females prenatally exposed to vehicle, 9 were injected with saline (VEH-SAL) before being placed in the reinforced compartment for 30 min. The other 9 females exposed prenatally to vehicle (VEH-MOR) and the 10 females prenatally exposed to flutamide (FLU-MOR) were injected with morphine (1 mg/kg) 1 min before being placed in the reinforced compartment.

3.2. Results: all females developed CPP after morphine administration

A clear change of preference was produced in both groups treated with morphine demonstrating that prenatally flutamide-treated females as well as females prenatally treated with vehicle have a functional reward system. No change in the time spent in the reinforced compartment between the pretest and test [$t(8)=-1.01$, $P=0.28$] was found in the VEH-SAL group. Fig. 1 illustrates the clear change of preference in the control [$t(8)=-4.98$, $P<0.01$] and flutamide groups [$t(9)=-2.42$, $P<0.05$] after the injection of morphine.

4. Experiment 2: conditioning with paced mating

4.1. Methods

We have previously shown that females receiving at least 10 paced intromissions develop CPP (see Introduction). In order to evaluate if flutamide-treated females needed fewer intromissions than control females to induce a reward state, 30 vehicle- and 10 flutamide-treated females were used. Ten control females were introduced in the reinforced compartment directly from their home cage without mating (VEH-0-Intro). Another 10 control females were gently introduced in the reinforced compartment after receiving between 10 and 15 intromissions with or without ejaculation (VEH>10-Intro). The remaining 10

Table 2
Feminine coital behavior of experimental females and masculine coital behavior of stimulus males

GROUP	ML	IL	EL	NM	NI	NE	III	LQ	LI
Experiment 2: paced mating									
VEH>10-Intro	51±16	78±19	439±76	10±2	11±1	0.7±0.1	49±7	96±1	1.7±0.05
VEH>9-Intro	20±4	48±8	256±33*	8±2	6±1**	1±0**	44±4	95±2	1.6±0.07
FLU>9-Intro	35±6	62±12	308±28	5±1	7±1**	1±0**	45±6	96±2	1.7±0.05
Experiment 3: non-paced mating									
VEH	47±18	73±21	372±68	12±2	11±1	0.8±0.1	43±6	99±1	1.8±0.07
FLU	46±22	69±29	429±84	9±1	9±1	1±0	48±9	98±1	1.7±0.04

ML—mount latency; IL—intromission latency; EL—ejaculation latency; III—interintromission interval; number of mounts (NM), intromissions (NI) and ejaculations (NE); lordosis quotient (LQ) and lordosis intensity (LI). Latencies are shown as seconds. The data represent the average of the three tests of coital behavior.

Mean±S.E.M. ANOVA followed by LSD Fisher test. *Different from vehicle with more than 10 intromissions (V>10Intro), $p<0.05$; **$p<0.01$.

control females were introduced in the reinforced compartment after receiving no more than 9 intromissions with or without ejaculation (VEH<9-Intro). Flutamide-treated females received no more than 9 intromissions (FLU<9-Intro) with or without an ejaculation before being placed in the reinforced compartment.

4.2. Results: flutamide-treated females needed fewer intromissions than control females to induce a reward state

Table 2 summarizes the sexual behavior displayed by stimulus males and experimental females. The ANOVA revealed significant differences in the number of intromissions (Group: $F(2,27)=15.9$, $p<0.001$), which was our criteria to differentiate the groups. With respect to pacing behavior, the ANOVA revealed differences in return latencies (groups, $F(2,27)=9.81$, $p<0.001$) and percent of exits (group, $F(2,27)=30.5$, $p<0.001$; behavioral pattern, $F(2,27)=3.1$, $p=0.048$) after mount, intromissions and ejaculation. Post hoc analysis showed that the percent of exits after ejaculation was increased with respect to percent of exits after mount and intromission in all groups (see Table 3). The return latency after ejaculation was increased with respect to the return latency after mount and intromission in the VEH>10-Intro group and in the FLU<9-Intro group. Differences in return latencies were not observed in the VEH<9-Intro group. Moreover, the ejaculation return latency in the VEH<9-Intro group was significantly reduced in comparison to the ejaculation return latency of the other 2 groups (Table 3).

Fig. 2 illustrates the clear increase of preference induced by pacing only in the control females with 10 or more intromissions [$t(9)=-3.25$, $P<0.01$] and in the flutamide-treated females with less than 9 intromissions [$t(9)=-2.55$, $P<0.05$]. No changes in the time spent in the reinforced compartment were observed in non-copulating females [$t(9)=-0.81$, $P=<0.43$] or in control females with less than 9 intromissions [$t(9)=-0.978$, $P=0.35$], nor was any change in the preference score observed in this control group [$t(9)=-1.71$, $P=0.11$].

Table 3
Return latency (in seconds) after mount (MRL), after intromission (IRL) and after ejaculation (ERL), and percent of exits after mount (%EM), intromission (%EI) or ejaculation (%EE) displayed by the females during paced mating (experiment 2)

Pacing behavior						
GROUP	MRL	IRL	ERL	%EM	%EI	%EE
VEH>10-Intro	17±3[a]	32±6[a]	68±24	15±5	22±6	65±11*
VEH>9-Intro	20±3	22±5	37±6[b]	20±7	30±8	80±9*
FLU>9-Intro	16±3[a]	29±6[a]	74±18	34±9	43±8	77±11[c]

The data represent the average of the three tests.
Mean±S.E.M. ANOVA followed by LSD Fisher test.
[a] Different from ERL in the same group, $p<0.01$.
[b] Different from the ERL of the other two groups, $p<0.05$.
[c] Different from %EM and %EI in the same group, $p<0.05$.
* $p<0.01$.

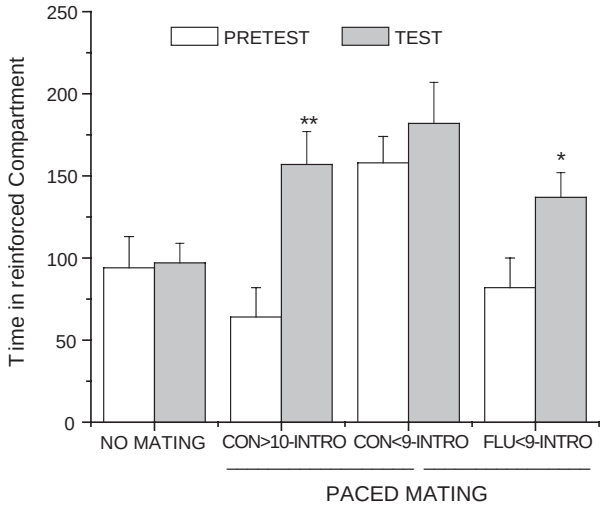

Fig. 2. Effect of paced mating on CPP. Time spend in the reinforced compartment (seconds) in control and flutamide-treated females allowed to paced their sexual interaction receiving different number of intromissions. Mean±S.E.M. *Different from pretest condition, $p<0.01$; **$p<0.01$.

5. Experiment 3: conditioning with non-paced mating

5.1. Methods

In experiment 2, we demonstrated that prenatal blockade of androgen receptors reduces the number of intromissions that females need to develop a reward state. In order to determinate if the prenatal blockade of androgen receptors inhibits the need to pace the coital contacts to induce a positive affective state, we evaluated if non-paced mating could induce a change in CPP. Ten vehicle (VEH)- and 10 prenatally flutamide-treated (FLU) females were used. Females were introduced in the non-preferred compartment after receiving 10 to 15 intromissions with or without ejaculation during the non-paced mating test.

5.2. Results: non-paced mating did not induce CPP

No differences were founded in masculine sexual behavior displayed by the stimulus males towards the VEH and FLU groups (Table 2). No change in preference was observed in the VEH [$t(9)=-2.22$, $P=0.062$] and FLU [$t(9)=-1.74$, $P=0.12$] groups not allowed to pace their sexual interaction (data not shown).

6. Discussion

Previous studies from our group have shown that the injection of the opiod antagonist naloxone prior to mating blocks CPP induced by paced mating, suggesting that the

reward state induced when females control the rate of sexual stimulation is mediated by opioids (Paredes and Martinez, 2001). The results of experiment 1 showed that morphine can induce a reward state in females prenatally treated with flutamide, suggesting that the opiod system is functional in these females and hence, that conditioned place preference induced by pacing behavior could be evaluated. The results of experiment 2 showed that flutamide-treated females were able to develop CPP after pacing; however, flutamide-treated females needed fewer intromissions than control females to induce a reward state. Previous observations showed that female rats need at least 10 paced intromissions to reach an affective positive state that induces CPP (Paredes and Alonso, 1997). Experiment 2 confirmed these observations; control females that received less than 9 intromissions with or without ejaculation showed no changes, whereas control females that received at least 10 intromissions with or without ejaculation showed a significant increase in the time in the reinforced compartment. Flutamide-treated females that received less than 9 intromissions with or without ejaculation also showed a significant increase in the time spent in the reinforced compartment, suggesting that the prenatal blockade of androgen receptors reduces the number of paced intromissions necessary to induce a reward state.

It is clearly established that cervical/vaginal stimulation is critical for the initiation of pregnancy (Adler, 1969; Lehmann and Erskine, 2004), but it is also well documented that this type of stimulation can be aversive for the female rat (Hardy and DeBold, 1972; Komisaruk and Whipple, 2000). In fact, vaginal stimulation can produce pleasure, pain and analgesia in rats and humans (see Komisaruk and Whipple, 2000 for a review). One of the possible adaptive significances of the analgesia induced by vaginal stimulation is that it could reduce the aversive sensory stimulation that occurs naturally during coitus. This in turn will increase the positive effects, pleasurable aspects, of the sensory stimulation (Komisaruk and Whipple, 2000). Pacing behavior allows the female to control the intensity of the stimulation (Erskine, 1989; Erskine and Baum, 1982b; Erskine et al., 1989; McClintock and Adler, 1978), reducing the aversive properties of mating and hence making sex rewarding (Martinez and Paredes, 2001; Paredes and Alonso, 1997; García-Horsman and Paredes, 2004). Individual differences in paced mating behavior have the potential to cause variation in fertility among female rats. Clemens et al. (1978) demonstrated that females receive androgens from adjacent males in prenatal life. This androgenic stimulation facilitates the expression, when adults, of masculine characteristics and can be blocked by flutamide (Gladue and Clemens, 1978). The ano-genital distance of prenatally flutamide-treated males from the same litters as the experimental females used in the present study was reduced. In addition to the feminization of the external genitalia, these males, from the same litters as our subjects, showed no intromission or ejaculation patterns (data not shown). These results confirm previous observations (Clemens et al., 1978; Imperato-McGinley et al., 1992; Dominguez-Salazar et al., 2002) and show that flutamide reached our subjects in uterus. The degree to which females are exposed to androgens during development is a potential explanation for individual differences in mating behavior, particularly in paced mating.

In the present experiment flutamide-treated females had a lordosis quotient and intensity similar to that displayed by control females. These results clearly indicate that our treatment with flutamide had no effect as an agonist of androgen receptors. Otherwise, a change in lordosis behavior would have been observed as is the case when females are prenatally treated with testosterone (Ward, 1969; Whalen and Edwards, 1967; Whalen and Robertson, 1968).

The display of pacing behavior is dependent upon different intensities of cervical–vaginal stimulation. If the perineal/vaginal area is anesthetized, females fail to pace (Bermant and Westbrook, 1966), and pelvic nerve transection increases the time females spend with a sexually active male (Emery and Whitney, 1985). In addition, cervical–vaginal stimulation alters subsequent behavior, such as an increase of the return latencies after each intromission (Erskine, 1989; Peirce and Nuttall, 1961). These observations suggest that motivation to mate decreases with the degree of cervical–vaginal stimulation; with higher stimulation, the time to return with the male is increased. It is interesting to note that control females with less than 9 intromissions did not increase the return latencies after ejaculation. Bermant and Westbrook (1966) suggested that continued vaginal distension or cervical stimulation provided by the deposition of vaginal plug after an ejaculation served to intensify the preceding stimulus. In our case, no differences in return latencies were observed within the less than 9 intromissions group (that included 1 ejaculation). Moreover, the ejaculation return latency of this group was significantly shorter that that observed in the control group with more than 10 intromissions and in the flutamide group with less than nine intromissions. These results suggest that a threshold of stimulation needs to be reached to observe a significant increase in the return latencies and early androgen levels might influence this threshold. For example, females treated with a small dose (5 μg) of testosterone propionate (TP) on day 3 postpartum showed delayed anovulatory syndrome but were genitally unaltered and maintained fertility during their young adult lives. However, during paced conditions, their III was increased (Gans and Erskine, 2003). The effect of TP treatment was to amplify the large effect that paced mating itself had on III, namely increased the return latencies after intromission. The authors explained this effect as a consequence of a decreased motivation to mate induced by testosterone. In agreement with this hypothesis, when we blocked the effect of testosterone with flutamide, females needed less than 9 intromissions to reach the stimulation threshold and develop CPP, whereas control females needed

more than 10 intromissions. These results are in agreement with early studies showing that under standard laboratory conditions, where females do not pace their sexual contacts, around 10 intromissions are needed to induce pseudopregnancy (Adler, 1969; Terkel and Sawyer, 1978) while under conditions where females can pace the sexual interaction, only 5 intromissions are necessary to induce pseudopregnancy.

We have previously demonstrated that only paced mating induces CPP (Paredes and Alonso, 1997), suggesting that during paced mating, female rats can dissociate the appetitive from the aversive components. In experiment 3, with non-paced mating, females were not able to develop CPP, suggesting that the prenatal blockade of androgen receptors did not modify the rewarding properties of mating in a non-paced mating test. To summarize, these experiments demonstrate that prenatal flutamide treatment reduced the number of intromissions needed to develop CPP only after paced mating, supporting our hypothesis that perinatal androgens can modulate the rewarding properties of mating in females. Further studies are needed to determine the role of perinatal aromatization of testosterone or mating-induced analgesia in the rewarding aspects associated with pacing behavior.

Acknowledgments

This project was supported by DGAPA IN227402 and CONACyT V40286M. We thank Pilar Galarza, Martin García, Lourdes Lara, Omar González, Leonor Casanova and Dorothy D. Pless for the technical assistance.

References

Adler NT. Effects of the male's copulatory behavior on successful pregnancy of the female rat. J Comp Physiol Psychol 1969;69:613–22.

Agmo A, Berenfeld R. Reinforcing properties of ejaculation in the male rat: role of opioids and dopamine. Behav Neurosci 1990;104:177–82.

Baum MJ. Effects of testosterone propionate administered perinatally on sexual behavior of female ferrets. J Comp Physiol Psychol 1976;90: 399–410.

Baum MJ. The ferret as a model for studying the sexual differentiation of behavioral and reproductive function. J Exp Zool Suppl 1990;4:213–4.

Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav 1976;7:105–38.

Bermant G, Westbrook WH. Peripheral factors in the regulation of sexual contact by female rats. J Comp Physiol Psychol 1966;61:244–50.

Blaustein JD, Erskine MS. Femenine sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. In: Pfaff DW, Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, editors. Hormones, brain and behavior, vol. 1. Academic Press; 2002. p. 139–213.

Brand T, Slob AK. Perinatal flutamide and mounting and lordosis behavior in adult female Wistar and Sprague–Dawley rats. Behav Brain Res 1991;44:43–51.

Calhoun JB. The ecology and sociology of the Norway Rat. Washington, D.C.: (U.S. Public Health Service Publication No. 1008) U.S. Government Printing Office, 1962.

Clemens LG, Gladue BA, Coniglio LP. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. Horm Behav 1978;10:40–53.

Dominguez-Salazar E, Portillo W, Baum MJ, Bakker J, Paredes RG. Effect of prenatal androgen receptor antagonist or aromatase inhibitor on sexual behavior, partner preference and neuronal Fos responses to estrous female odors in the rat accessory olfactory system. Physiol Behav 2002;75:337–46.

Emery DE, Whitney JF. Effects of vagino-cervical stimulation upon sociosexual behaviors in female rats. Behav Neural Biol 1985;43: 199–205.

Erskine MS. Solicitation behavior in the estrous female rat: a review. Horm Behav 1989;23:473–502.

Erskine MS, Baum MJ. Effects of paced coital stimulation on termination of estrus and brain indoleamine levels in female rats. Pharmacol Biochem Behav 1982a;17:857–61.

Erskine MS, Baum MJ. Plasma concentrations of testosterone and dihydrotestosterone during perinatal development in male and female ferrets. Endocrinology 1982b;111:767–72.

Erskine MS, Kornberg E, Cherry JA. Paced copulation in rats: effects of intromission frequency and duration on luteal activation and estrus length. Physiol Behav 1989;45:33–9.

Fang J, Chung YW, Clemens LG. Relation of Fos-IR expression in the pelvic ganglion to sexual behavior in laboratory rats. Behav Neurosci 2000;114:543–52.

Fiber JM, Swann JM. Testosterone differentially influences sex-specific pheromone-stimulated Fos expression in limbic regions of Syrian hamsters. Horm Behav 1996;30:455–73.

Fuh VL, Stoner E. Androgens inhibitor/antiandrogens. In: Knobil E, Neill JDEncyclopedia of reproductionvol. 1. San Diego, CA: Academic Press; 1998. p. 166–73.

Gans S, Erskine MS. Effects of neonatal testosterone treatment on pacing behaviors and development of a conditioned place preference. Horm Behav 2003;44:354–64.

García-Horsman P, Paredes RG. Dopamine antagonists do not block conditioned place preference induced by paced mating behavior in female rats. Behav Neurosci 2004;118:356–64.

Gladue BA, Clemens LG. Androgenic influences on feminine sexual behavior in male and female rats: defeminization blocked by prenatal antiandrogen treatment. Endocrinology 1978;103:1702–9.

Hardy DFA, DeBold JF. Effects of coital stimulation upon behavior of the female rat. J Comp Physiol Psychol 1972;78:400–8.

Hughes AM, Everitt BJ, Herbert J. Comparative effects of preoptic area infusions of opioid peptides, lesions and castration on sexual behaviour in male rats: studies of instrumental behaviour, conditioned place preference and partner preference. Psychopharmacology (Berl) 1990;102:243–56.

Imperato-McGinley J, Sanchez RS, Spencer JR, Yee B, Vaughan ED. Comparison of the effects of the 5 alpha-reductase inhibitor finasteride and the antiandrogen flutamide on prostate and genital differentiation: dose–response studies. Endocrinology 1992;131:1149–56.

Komisaruk BR, Whipple B. How does vaginal stimulation produce pleasure, pain and analgesia? In: Fillingm RBSex, gender and pain, progress in pain research and managementvol. 17. Seattle: IASP Press; 2000. p. 109–34.

Lee JW, Erskine MS. Changes in pain threshold and lumbar spinal cord immediate-early gene expression induced by paced and nonpaced mating in female rats. Brain Res 2000;861:26–36.

Lehmann ML, Erskine MS. Induction of pseudopregnancy using artificial VCS: importance of lordosis intensity and prestimulus estrous cycle length. Horm Behav 2004;45:75–83.

Madlafousek J, Hlinak Z. Sexual behavior of the female laboratory rat: inventory, patterning, and measurement. Behaviour 1977;63:129–74.

Martinez I, Paredes RG. Only self-paced mating is rewarding in rats of both sexes. Horm Behav 2001;40:510–7.

McClintock MK, Adler NT. The role of the female during copulation in wild and domestic Norway rats (*Rattus norvergicus*). Behaviour 1978;67:67–96.

Mehrara BJ, Baum MJ. Naloxone disrupts the expression but not the acquisition by male rats of a conditioned place preference response for an oestrous female. Psychopharmacology (Berl) 1990;101:118–25.

Miller RL, Baum MJ. Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration. Pharmacol Biochem Behav 1987;26:781–9.

Noble RG. Mounting in female hamsters: effects of different hormone regimens. Physiol Behav 1977;19:519–26.

Paredes RG, Alonso A. Sexual behavior regulated (paced) by the female induces conditioned place preference. Behav Neurosci 1997;111: 123–8.

Paredes RG, Martinez I. Naloxone blocks place preference conditioning after paced mating in female rats. Behav Neurosci 2001;115: 1363–7.

Paredes RG, Vazquez B. What do female rats like about sex? Paced mating. Behav Brain Res 1999;105:117–27.

Peirce JT, Nuttall RL. Self-paced sexual behavior in the female rat. J Comp Physiol Psychol 1961;54:310–3.

Pfaus JG, Smith WJ, Coopersmith CB. Appetitive and consummatory sexual behaviors of female rats in bilevel chambers: I A correlational and factor analysis and the effects of ovarian hormones. Horm Behav 1999;35:224–40.

Terkel J, Sawyer CH. Male copulatory behavior triggers nightly prolactin surges resulting in successful pregnancy in rats. Horm Behav 1978;11: 304–9.

Tobet SA, Baum MJ. Role for prenatal estrogen in the development of masculine sexual behavior in the male ferret. Horm Behav 1987;21: 419–29.

Vomachka AJ, Lisk RD. Androgen and estradiol levels in plasma and amniotic fluid of late gestational male and female hamsters: uterine position effects. Horm Behav 1986;20:181–93.

Ward IL. Differential effect of pre- and post-natal androgen on the sexual behavior of intact and spayed female rats. Horm Behav 1969;1:25–36.

Whalen RE, Edwards DA. Hormonal determinants of the development of masculine and feminine behavior in male and female rats. Anat Rec 1967;157:173–80.

Whalen RE, Robertson RT. Sexual exhaustion and recovery of masculine copulatory behavior in virilized female rats. Psychon Sci 1968;11: 319–20.

Yang LY, Clements LG. MPOA lesions affect female pacing of copulation in rats. Behav Neurosci 2000;114:1191–202.