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Neuroendocrine and reproductive aspects of adult male rats exposed neonatally to an antiestrogen

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Abstract

The present study was designed to investigate the effects of a single dose of an estrogen antagonist–clomiphene–during neonatal life, on later neuroendocrine system and reproductive performance. Immediately after birth, male pups received clomiphene citrate (s.c.). At adulthood, although testosterone levels and wet weights of reproductive organs were not altered, the treatment induced an increased number of spermatozoa and a delay in the transit time in the cauda epididymis. Additionally, there was impairment of sexual behavior evidenced by a delay in the latencies to the first mount and first intromission. Treated rats also showed decreased dopaminergic and serotonergic neurotransmissions in the hypothalamus and decreased dopaminergic neurotransmission in the striatum. The decreased dopaminergic activity could be related to the lower sexual motivation observed. These results indicate the necessity of preventing exposure to drugs that may impair sexual differentiation, which can compromise later mating success as well as the capacity to generate descendants.

Keywords: Antiestrogen; Clomiphene; Hypothalamus; Neurochemistry; Rat; Sexual behavior; Sexual differentiation; Spermatozoa; Striatum; Testosterone

1. Introduction

Brain sexual differentiation occurs in the perinatal period after an abrupt discharge of testicular testosterone in males. In male rats, testosterone surges markedly on days 18–19 of gestation (Weisz and Ward, 1980; Ward and Weisz, 1984) and again during the first few hours following parturition (Corbier et al., 1978). Thus, early exposure to androgen from developing testes results in masculinization and defeminization of the brain. The former entails permanent actions that support male typical copulatory behaviors and patterns of gonadotropin secretion. Actually, it is not testosterone per se that is responsible for masculinizing the brain (Roselli and Kosterman, 1998). This process requires the conversion of androgen to estrogen and the neural aromatization of androgens to estrogens is known to be a critical step in the development and adult expression of male sexual behavior in a variety of species (Freeman and Rissman, 1996; Lephart, 1996). During this period of brain sexual differentiation, testosterone or its metabolites are fundamental for masculinization and defeminization of sexual behavior, for the establishment of gonadotropin secretion patterns, and also for various morphological indices. Alterations in the process of hypothalamic sexual differentiation, if present, generally are perceived only at puberty or in adult reproductive life (Piffer and Pereira, 2004).

Therefore, the aim of the present study was to determine the effects of an antiestrogen–clomiphene–administration during the period of brain sexual differentiation on neuroendocrine and sexual aspects in adult male rats.

2. Materials and methods

2.1. Animals and experimental protocol

Wistar rats were used as the parent generation. They were kept in a controlled environment with temperature at 25±1 °C; humidity of 55±5%; 12-h light/dark cycle (lights on at

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6:00 a.m.) and had free access to regular lab chow (estrogen-free) and tap water. Virgin female rats (200±10 g) were mated overnight. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning and was considered day 1 of gestation. On GD22, all dams ($n=10$) were weighed, anaesthetized with sodium pentobarbital (40 mg/kg, i.p.), and laparotomized to obtain male pups, which were divided according to treatment, as described below. The experimental procedures were not done at the same time, since the animals used for each evaluation was obtained from different mothers, so not necessarily born at the same day.

- control group: immediately after birth, 26 male pups obtained from different dams received, s.c., 0.1 ml of physiologic solution (0.9% NaCl—vehicle).
- clomiphene group: immediately after birth, 26 male pups obtained from different dams received, s.c., clomiphene citrate (2 mg/kg) dissolved in 0.1 ml of physiologic solution (Shughrue et al., 1997).

The pups of both groups were immediately fostered to recipient dams (8 pups/recipient dams) that had not been manipulated during the gestation and delivered on the same day. The pups were culled to six males and two females to ensure the presence of both sexes in the litters. They were left with each dam until weaning (23 days of age). At this age, the male pups were housed in collective polypropylene cages ($32\times40\times18$ cm^3), 4 animals/cage until 75 days of age. For each set of experiments, a maximum of two male siblings was taken from each litter in order to avoid "litter effects".

The animals used in this study were maintained in accordance with Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and approved by the Bioscience Institute/UNESP Ethical Committee for Animal Research (Protocol number: 065/03). The experimental protocol is diagramed in Fig. 1.

2.2. Body weight and wet weights of the testis, epididymis, prostate, and seminal vesicle

Ten male pups per group were weighed and anaesthetized with sodium pentobarbital (40 mg/kg, i.p.). Blood samples were collected and the animals were killed by decapitation. One testis, one epididymidis, ventral prostate gland and seminal vesicle were removed for wet-weight determination.

2.3. Plasmatic testosterone quantification

Blood from the abdominal aorta was collected, centrifuged (2500 rpm for 20 min at 2 °C), and the plasma stored at −20 °C until assayed. Plasma testosterone level was measured by radioimmunoassay using Coat-A-Count Total Testosterone Kit (Diagnostic Products Co., Los Angeles, CA) according to manufacturer's instructions. The assay detection limits was 0.04 ng/ml.

2.4. Spermatozoa quantification

Spermatozoa were quantified in both groups (9 males in control group and 10 males in clomiphene group). Homogenization-resistant testicular spermatids in the testes and sperm in the caput/corpus epididymidis and cauda epididymidis were enumerated as described previously (Robb et al., 1978). Daily sperm production per testis (i.e. DSP) was determined by dividing the total number of homogenization-resistant spermatids per testis by 6.1 days, the number of days of a seminiferous cycle in which these spermatids are present. Transit times through the caput/corpus epididymidis and cauda epididymidis were calculated by dividing the number of sperm within each of these regions by the DSP.

2.5. Sexual behavior evaluation

Sexually inexperienced male pups (9 males in control group and 10 males in clomiphene group) were observed under red-light illumination during the dark phase of their cycle. For the test, female rats in their estrus phase (induced by estradiol benzoate −20 μg/kg, i.p., 24 h before test) were used (Arteche et al., 1997). Each male was placed into a Plexiglass cage and after 5 min, the female was introduced. During 30 min, the following parameters were recorded: mount (the male normally mounts from the rear, sometimes placing his forelegs over the female's back, and makes rapid anteroposterior pelvic thrusts), intromission (vaginal penetration, this behavior starts with a mount, but suddenly the male makes a deep thrust forward and stops pelvic thrusting, then vigorously withdraws and always licks his genitals), and ejaculation (stars with an intromission, but after vaginal penetration the male remains on the female for 1–3 s) latencies; number of mounts and intromission until the first ejaculation; mount and intromission latencies after the first ejaculation; and number of postejaculatory mounts and

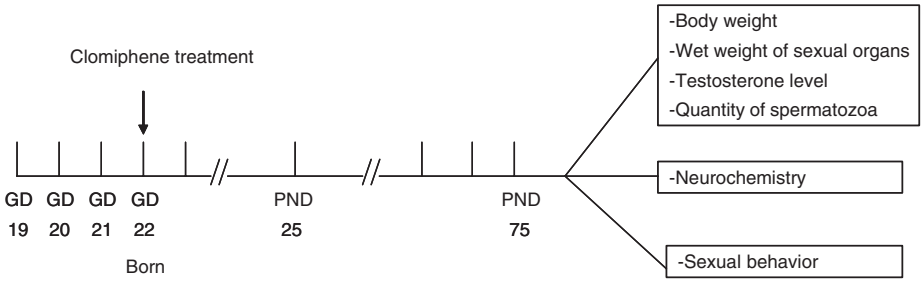

Fig. 1. Diagram of the experimental design. GD: gestational day; PND: postnatal day.

Table 1
Plasma testosterone concentration, body weight, and wet weight of organs from control and clomiphene-treated adult male rats

Parameters	Experimental group	
	Control	Clomiphene
Testosterone levels (ng/ml)	3.72±0.52 (8)	4.92±0.95 (8)
Body weight (g)	367.03±5.50 (10)	389.95±10.87 (10)
Organ weight (g)		
Testis wet weight	1.53±0.04 (10)	1.57±0.04 (10)
Epididymis wet weight	0.50±0.01 (10)	0.53±0.01 (10)
Prostate wet weight	0.36±0.03 (10)	0.37±0.02 (10)
Seminal vesicle wet weight (with secretion)	0.52±0.03 (10)	0.55±0.04 (10)
Seminal vesicle wet weight (without secretion)	0.19±0.01 (10)	0.18±0.01 (10)

Data are means±S.E.M.
Numbers in parentheses represent the number of animals per group.
No significant difference was found ($p>0.05$ by Student's t-test).

intromissions. If a male did not mount or intromit within 10 min, the evaluation was ended and the male was considered sexually inactive (Agmo, 1997).

2.6. Neurochemical evaluation

Immediately after decapitation of the other male pups, entire hypothalamus and striatum were excised as rapidly as possible (not more than 3 min) and were weighed and frozen on dry ice. The samples were homogenized in 0.1 M perchloric acid containing 1.1 mM sodium metabisulphite and 0.54 mM disodium EDTA by sonication and centrifuged for 30 min at 11,200×g. The supernatant was stored frozen at − 80 °C until analysis.

Norepinephrine (NE), dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as serotonin (5-HT) and its metabolites 5-hydroxyindoleacetic acid (5-HIAA) were measured using an HPLC system (model 6A, Shimatzu, Kyoto, Japan) with an electrochemical detector (model 6A, Shimatzu, Kyoto, Japan). For the analysis, samples were loaded into a sample injector (valve for 20 μl), and the mobile phase was delivered at a constant rate of 1 ml/min through a C18 analytical column (Shimpak, ODS, Kyoto, Japan) placed in a column heater (35 °C). The amperometric potential was set to 0.83 V with reference to an Ag–Ag–Cl reference electrode. The mobile phase (pH=2.9) consisted of 0.02 M disodium phosphate, 0.02 M citrate, 0.11 mM disodium EDTA, 2.75 mM heptanosulfonic acid, and 10% methanol. The signal from the detector was recorded by an integrator (Chromatopac, Shimatzu, Kyoto, Japan). The runtime for each sample was 25 min. The detection limits were 0.02 ng for HVA and 0.002 ng for DA, DOPAC, 5-HT and 5-HIAA.

The concentrations of the neurotransmitters were expressed as ng/g of tissue. DA and 5-HT metabolic rates were expressed as the ratios DOPAC/DA, HVA/DA and 5-HIAA/5-HT.

2.7. Statistical analysis

The results were submitted to descriptive statistics for determination of normal distributions of data in order to verify if there were any discrepant data. After this, the Student's t-test was employed, with the results considered significant at $p<0.05$.

3. Results

3.1. Body weight and wet weights of testis, epididymis, ventral prostate, and seminal vesicle

As shown in Table 1, neonatal clomiphene treatment did not alter either the body weight of the animals or the wet weight of testis, epididymis, prostate, and seminal vesicle.

3.2. Plasmatic testosterone quantification

As shown in Table 1, males treated neonatally with clomiphene did not present alteration in plasmatic testosterone level when compared to control pups.

3.3. Spermatozoa quantification

Adult male rats treated neonatally with clomiphene presented, compared to control group, an increase in the mean of germ cells per organ [$t(17)=3.833$, $p=0.0013$] and g/organ [$t(17)=2.210$, $p=0.041$] in the cauda as well as a delay in sperm transit time through the cauda [$t(17)=3.740$, $p=0.0016$], as shown in Table 2.

3.4. Male sexual behavior evaluation

Data from male sexual behavior evaluation are shown in Table 3. Treatment with clomiphene induced a delay in the

Table 2
Mean number of spermatids and daily sperm production in the testes and mean number of sperm in the caput/corpus and cauda epididymidis and sperm transit time from control and clomiphene-treated adult male rats

Parameters	Experimental groups	
	Control (9)	Clomiphene (10)
Number of spermatids (10^6/testis)	170.63±6.20	165.81±8.64
Number of spermatids (10^6/g/testis)	127.65±5.46	130.88±2.15
DSP	27.97±1.01	27.17±1.41
Number of spermatozoa×10^6/caput+corpus of epididymis	99.92±9.18	77.37±6.64
Number of spermatozoa×10^6/g/caput+corpus of epididymis	388.13±22.38	336.50±23.45
Number of spermatozoa×10^6/cauda of epididymis	128.26±6.92	185.04±12.57**
Number of spermatozoa×10^6/g/cauda of epididymis	751.10±33.32	859.40±33.50*
Sperm transit time (days) through caput/corpus of epididymis	3.57±0.30	2.85±0.21
Sperm transit time through cauda of epididymis (days)	4.61±0.25	6.91±0.53**

DSP: daily sperm production (number of spermatids×10^6/testis/day).
Data are means±S.E.M. Numbers in parentheses represent the number of animals per group.
* $p<0.05$ compared to the control group (Student's t-test).
** $p<0.01$ compared to the control group (Student's t-test).

Table 3
Effects of neonatal treatment with clomiphene on sexual behavior from control and clomiphene-treated adult male rats

Parameters	Groups	
	Control	Clomiphene
Latency to first mount (s)	64.22±14.83 (9/9)	193±26.38**
Number of mounts without intromission	4.22±1.62 (9/9)	1.0±0 (3/10)
Latency to first intromission (s)	83.00±19.00 (9/9)	193.0±26.38* (3/10)
Number of intromission	29.11±1.85 (9/9)	31.0±1.0 (3/10)
Latency to first ejaculation (s)	1155.00±126.12 (9/9)	1074.0±240.33 (3/10)
Postejaculatory mount latency (s)	1315.87±153.98 (8/9)	1328±243.69 (3/10)
Postejaculatory intromission latency (s)	1316.37±153.66 (8/9)	1330.33±244.02 (3/10)
Number of de postejaculatory intromission	15.12±2.31 (8/9)	14.0±4.6 (3/10)
Number of ejaculation	1.55±0.24 (9/9)	2.0±0.5 (3/10)

Data are means±S.E.M.
Numbers in parentheses represent the number of animals per group.
* $p<0.05$ compared to the control group (Student's t-test).
** $p<0.01$ compared to the control group (Student's t-test).

latencies to the first mount [$t(10)=4.376$, $p=0.0014$] and to the first intromission [$t(10)=2.995$, $p=0.0135$]. The other parameters were not significantly altered. Moreover, in the treated group 7 out of 10 animals not presented male sexual behavior. These animals were discharged from the analysis of these parameters and were considered sexually inactive.

3.5. Neurochemical evaluation

There was an increase in NE levels [$t(10)=2.295$, $p=0.0446$] and a decrease in DOPAC [$t(10)=3.246$, $p=0.0088$], 5-HT [$t(10)=2.239$, $p=0.0491$] and 5-HIAA [$t(10)=3.392$, $p=0.0069$] levels in the hypothalamus of male rats treated neonatally with clomiphene (Table 4). However, in the striatum, males treated with clomiphene presented higher DA [$t(11)=2.662$, $p=0.0221$], 5-HT [$t(11)=2.398$, $p=0.0354$] and a decrease in DOPAC/DA [$t(11)=3.235$, $p=0.0079$] levels when compared to control pups (Table 5).

Table 4
Hypothalamic monoamine levels (ng/g) from control and clomiphene-treated adult male rats

Neurotransmitters	Groups	
	Control (6)	Clomiphene (6)
NE	736.66±28.37	884.66±57.89*
DA	274.16±25.98	271.16±18.60
DOPAC	27.33±1.76	19.83±1.49**
HVA	16.83±1.01	15.50±0.50
DOPAC/DA	0.105±0.01	0.074±0.006
HVA/DA	0.065±0.007	0.058±0.004
5-HT	2090.00±43.08	1987.33±15.70*
5-HIAA	917.83±32.84	734.83±42.80**
5-HIAA/5-HT	0.438±0.01	0.369±0.02*

Norepinephrine (NE); dopamine (DA); 3,4-dihydroxyphenylacetic acid (DOPAC); serotonin (5-HT); 5-hydroxyindoleacetic acid (5-HIAA).
Data are means±S.E.M. Numbers in parentheses represent the number of animals per group.
* $p<0.05$ compared to the control group (Student's t-test).
** $p<0.01$ compared to the control group (Student's t-test).

Table 5
Striatal monoamine levels (ng/g) from control and clomiphene-treated adult male rats

Neurotransmitters	Groups	
	Control (7)	Clomiphene (6)
NE	28.48±2.75	27.30±3.6
DA	7115.71±229.35	7860.00±137.60*
DOPAC	551.14±23.39	550.00±6.83
HVA	505.71±29.50	546.66±16.05
DOPAC/DA	0.077±0.001	0.070±0.001**
HVA/DA	0.071±0.004	0.069±0.002
5-HT	858.57±27.81	966.66±36.39*
5-HIAA	811.42±30.50	816.66±16.66
5-HIAA/5-HT	0.934±0.02	0.850±0.03

Norepinephrine (NE); dopamine (DA); 3,4-dihydroxyphenylacetic acid (DOPAC); homovanillic acid (HVA); serotonin (5-HT); 5-hydroxyindoleacetic acid (5-HIAA).
Data are means±S.E.M. Numbers in parentheses represent the number of animals in each group.
* $p<0.05$ compared to the control group (Student's t-test).
** $p<0.01$ compared to the control group (Student's t-test).

4. Discussion

Clomiphene–an estrogen drug that mixes agonist and antagonist activity–has been used as estrogen receptor blocker in the study of molecular mechanisms of rat brain sexual differentiation (Giannakopoulou et al., 2001). In the present study, male rats exposed neonatally to clomiphene exhibited no alteration in adulthood testosterone level as well as on wet weights of the testis, epididymis, ventral prostate or seminal vesicle. These findings are in agreement with the literature that reports no alterations in testosterone and LH concentrations in blood plasma as well as on body and accessory sexual glands weights following the antiestrogen ICI182,780 treatment (Oliveira et al., 2002). A preliminary study in our laboratory showed the importance of estrogen in the process of brain sexual differentiation in males and suggests a non-complete defeminization or masculinization of the hypothalamus in males rats exposed neonatally to clomiphene (Pereira et al., 2003).

Moreover, male rats treated with clomiphene at birth showed an increased spermatozoa quantity and a delay in the transit time in the cauda epididymis during adulthood. The epididymis is an organ in which sperm undergo maturation, storage, and transport prior to ejaculation. Studies using both chemical and surgical denervation of reproductive organs have confirmed that autonomic innervation plays a significant role in epididymal functions. In this way, in rats, the low level guanethidine or surgically induced sympathectomy delays the transit of sperm through the cauda but does not compromise sperm quality in the distal cauda epididymis or impair the ability of those sperm to fertilize ova in vivo (Kempinas et al., 1998). The sperm

transport is accomplished by contractility of this organ and estrogen is involved in this process; however, it is unclear whether the effects of estrogen on the epididymis are direct or indirect (Hess et al., 2001). Studies have shown that estrogen treatment, produces harmful effects on the epididymis and reduces the fertilizing ability of epididymal sperm even when it does not imbalance testosterone level (Lubicz-Nawrocki, 1974). So, in the present study, the results suggest that the increased number of spermatozoa could be related to a delay in the transit time observed, which could be induced by a reduced contractility of the duct.

Regarding sexual behavior, neonatal clomiphene treatment disrupted it, as demonstrated by delayed latencies to the first mount and first intromission. This result is in accordance to a study that demonstrated absence of sexual activity in male rats treated with tamoxifen–another antiestrogen drug–after birth. It is noteworthy the extreme sensitivity of the receptor–signal transduction system in the hypothalamic sexual nuclei during this early period of life, which disturbance evoked a complete inhibition of sexual activity instead of provoking only deficiencies (Csaba and Karabélyos, 2001). Additionally, 70% of the treated males were inactive male-typical sexual behavior, demonstrating the necessity of estrogen presence during the neonatal period.

Moreover, male sexual behavior in adult mammals is also modulated by testosterone (Robbins, 1996); and it requires normal functioning of the hypothalamic–pituitary–testicular axis (Agmo, 1997). Although, in the present study, no alteration was observed in testosterone levels, there was an impaired sexual behavior suggesting no alterations in neurotransmitters and/or SNC areas involved in sexual behavior in treated animals. Thus, low testosterone levels in adult males may not be the only factor related to behavioral abnormalities. In fact, a decrease in the brain response to androgenic activation of male sexual behavior has also been involved in behavioral abnormalities. One step in the translation of long-term steroid effects into rapid sexual behavioral events is probably a change in the release or effectiveness of one or more neurotransmitters (Hull et al., 1999). An early modification of neuronal reactivity to hormones and neurotransmitters appears to be a key point of genetic imprinting in the developing brain (Reznikov et al., 1999).

In the present study, neonatal clomiphene treatment led to neurochemical alterations in the hypothalamus and striatum of adult male rats. Increased NE levels were observed in the hypothalamus, a decreased DOPAC levels without any changes in DA levels, suggesting a decreased release of this neurotransmitter; and decreased 5-HT and 5-HIAA levels, indicating a reduced activity of the serotonergic system. The stimuli from a receptive female or copulation itself leads to the release of dopamine in three principal integrative sites that control sexual motivation and both genital and somatomotor responses in male rats. The medial preoptic area is the most important region for male sexual behavior, as it modifies the processing of sensory stimuli, receives indirect sensory input from virtually every sensory modality and sends reciprocal connections back to those sources (Markowski et al., 1994). Therefore, it is critical to the initiation of copulation. The control of copulatory events also depends on differences in levels of extracellular DA and its action on different families of dopaminergic receptors (Markowski et al., 1994; Hull et al., 1986). Thus, dopamine has a central role in the translation of long-term steroid effects into rapid behavioral events, facilitating sexual behavior. The impairment of male sexual behavior observed in this study was characterized by alterations in sexual appetite (mount and intromission latencies), which may have been induced by the decreased dopaminergic activity observed in the hypothalamus.

The lower sexual motivation observed in the present study may be related to a delay in mount and intromission latencies in the male rats treated with clomiphene. Thus, the increased intromission latencies and decreased numbers of intromissions in the clomiphene-treated males that actually copulated could also indicate a primary erectile dysfunction.

Although less studied, 5-HT also plays a role in sexual behavior. Serotonin is regarded as an inhibitory neurotransmitter and, therefore, the decrease of serotonergic activity facilitates sexual behavior (Bitran and Hull, 1987). Different subtypes of 5-HT receptors appear to mediate the inhibitory effects of 5-HT on erection and on ejaculation. 5-HT is released in the anterior lateral hypothalamus at time of ejaculation (Hull et al., 2004). In contrast to the effects of 5-HT_{2C} agonists, stimulation of 5-HT_{1A} receptors in the MPOA facilitated ejaculation (Matuszewich et al., 1999). Therefore, the facilitative effects of the 5-HT_{1A} agonist may be mediated in part through its increase in extracellular DA in the medial preoptic area (MPOA). Although, in the present study a decreased serotonergic activity was observed, the sexual behavior of male rats was not facilitated. Probably the decrease in serotonergic system plus a decrease in the release extracellular DA via DOPAC in the hypothalamus could be involved with inhibition of the sexual behavior. Thus, the central nervous system connections are complex and the knowledge of the interactions among neurotransmitters is yet incipient. So, we can not explain this result by now.

In the striatum, male rats treated with clomiphene presented increased DA and 5-HT levels and decreased DOPAC/DA levels, which also indicates a decreased dopaminergic activity. The nigrostriatal system contributes to the execution of consummatory movements, pursuit of the female and mount (Robbins and Everitt, 1992), reflecting a primarily motor activation. These parameters of sexual behavior depend on steroid hormones that act within the central nervous system, modifying neuronal excitability and several neurotransmitter systems in specific brain structures (Hull et al., 1999). Although, the neurochemistry result in the present study has demonstrated a decreased dopaminergic activity in the striatum, male rats did not present alteration in motor function as observed by the normal number of mounts and intromissions.

Considering the results of the present study it can be concluded that the neonatal clomiphene has a life-long influence on the spermatozoa quantification, sexual behavior, and brain neurotransmitters in male pups. These results also indicate the necessity of preventing exposure to drugs that may

impair sexual differentiation, which can compromise later mating success as well as the capacity to generate descendants.

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References

Agmo A. Male rat sexual behavior. Brain Res Protoc 1997;1:203–9.

Arteche E, Strippoli G, Loirand G, Pacaud P, Candenas L, Moltó J, et al. An analysis of the mechanisms involved in the okadaic acid induced contraction of the estrogen-primed rat uterus. J Pharmacol Exp Ther 1997;282:201–7.

Bitran D, Hull EM. Pharmacological analysis of male rat sexual behavior. Neurosci Biobehav Rev 1987;11:365–89.

Corbier P, Kerdelhue B, Picon R, Roffi J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology 1978;103:1985–91.

Csaba G, Karabèlyos C. The effect of a single neonatal treatment (hormonal imprinting) with the antihormones, tamoxifen and mifepristone on the sexual behavior of adult rats. Pharmacol Res 2001;43(6):531–3.

Freeman LM, Rissman EF. Neural aromatization and the control of sexual behavior. Trends Endocrinol Metab 1996;7:334–8.

Giannakopoulou M, Bozas E, Philippidis H, Stylianopoulou F. Protooncogene c-fos involvement in the molecular mechanism of rat brain sexual differentiation. Neuroendocrinology 2001;73:387–96.

Hess RA, Zhou Q, Nie R, Oliveira C, Cho H, Nakai M, et al. Estrogens and epididymal function. Reprod Fertil Dev 2001;13:273–83.

Hull EM, Britan D, Pehek EA, Warner RK, Band LC, Holmes GM. Dopaminergic control of male sex behavior in rats: effects of intracerebrally infused agonist. Brain Res 1986;370:73–81.

Hull EM, Lorrain DS, Du J, Matuszewich L, Lumley LA, Putnam SK, et al. Hormone–neurotransmitter interactions in the control of sexual behavior. Behav Brain Res 1999;105:105–16.

Hull EM, Muschamp JW, Sato S. Dopamine and serotonin: influences on male sexual behavior. Physiol Behav 2004;83:291–307.

Kempinas WG, Suarez JD, Roberts NL, Strader LF, Ferrell J, Goldman JM, et al. Fertlility of rat epididymal sperm after chemically and surgically induced sympathectomy. Biol Reprod 1998;59:897–904.

Lephart ED. A review of brain aromatase cytochrome P450. Brain Res Rev 1996;22:1–26.

Lubicz-Nawrocki CM. The inhibition of fertilizing ability of epididymal spermatozoa by the administration of oestradiol benzoate to testosterone-maintained hypophysectomized or castrated hamsters. J Endocrinol 1974;61:133–8.

Markowski VP, Lumeley LA, Moses J, Hull EM. D_1 agonist in the MPOA facilitates copulation in male rats. Pharmacol Biochem Behav 1994;47:483–6.

Matuszewich L, Lorrain DS, Trujillo R, Domingues J, Putnam SK, Hull EM. Partial antagonism of 8-OH-DPAT's effects on male rat sexual behavior with D2, but not a 5-HT1A, antagonist. Brain Res 1999;820:55–62.

Oliveira AC, Zhou Q, Carnes K, Nie R, Kuehl DE, Jackson GL, et al. ER function in the adult male rat: short- and long-term effects of the antiestrogen ICI182,780 on the testis and efferent ductules, without changes in testosterone. Endocrinology 2002;143:2399–409.

Pereira OCM, Coneglian-Marise MSP, Gerardin DCC. Effects of neonatal clomiphene citrate on fertility and sexual behavior in male rats. Comp Biochem Physiol A 2003;134:545–50.

Piffer RC, Pereira OCM. Reproductive aspects in female rats exposed prenatally to hydrocortisone. Comp Biochem Physiol C 2004;139:11–6.

Reznikov AG, Nosenko ND, Tarasenko LV. Prenatal stress and glucocorticoid effects on the developing gender-related brain. J Steroid Biochem Mol Biol 1999;69:109–15.

Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J Reprod Fertil 1978;54:103–7.

Robbins A. Androgens and male sexual behavior, from mice to men. Trends Endocrinol Metab 1996;7:345–50.

Robbins TW, Everitt BJ. Functions of dopamine in the dorsal and ventral striatum. Semin Neurosci 1992;4:119–28.

Roselli CE, Kosterman SA. Sexual differentiation of aromatase activity in the rat brain: effects of perinatal steroid exposure. Endocrinology 1998; 139:3193–201.

Shughrue PJ, Lane MV, Merchenthaler I. Regulation of progesterone receptor messenger ribonucleic acid in the rat medial preoptic nucleus by estrogenic and antiestrogenic compounds: an in situ hybridization study. Endocrinology 1997;138:5476–84.

Ward IL, Weisz J. Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology 1984;114:1635–44.

Weisz J, Ward IL. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 1980;106:306–16.