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PRENATAL INFLUENCES OF ANDROGENS AND RELATED METABOLITES UPON THE DEVELOPMENT OF PACED SEXUAL BEHAVIOR IN THE FEMALE RAT (*RATTUS NORVEGICUS*)

By

Gary Michael Lange

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ABSTRACT

PRENATAL INFLUENCES OF ANDROGENS AND RELATED METABOLITES UPON THE DEVELOPMENT OF PACED SEXUAL BEHAVIOR IN THE FEMALE RAT (*RATTUS NORVEGICUS*)

By

Gary Michael Lange

Traditional views of adult sex behavior in rats have emphasized the differences found between males and females. Males have been viewed as more active in their sex behavior, whereas females have been viewed as more *reactive*. More recently, female sex behavior has been examined for its active components in order to more closely compare the behavior of both sexes.

In the temporal regulation of copulatory behavior, both sexes experience a refractory period after ejaculation, and both regulate the intervals between non-ejaculatory intromissions. In sex behavior tests where the male is given free access to the female, his temporal regulation of intromissions is generally faster than in tests where the female is able to escape from the male after each intromission. Additionally, following an ejaculation, a female's refractory period is typically shorter than the male's. In the discussion that follows, a testing paradigm allowing the female to pace her behaviors through escape from the male is used to discern if sexually dimorphic behaviors in the temporal regulation of copulation

result from differential exposure to steroid hormones during early development.

With early prenatal exposures to androgens or the aromatase enzyme inhibitor ATD, virilization was seen at birth. Additionally, intromission rates were increased in adulthood in females exposed to androgens or ATD. Refractory periods were diminished or abolished in females exposed to androgens or ATD.

These findings suggest that the role of testosterone and its metabolites can have profound effects in the temporal patterning of sexual behavior in female rats when this exposure occurs during prenatal development or early in neonatal life.

To my mother, Buelah E. Lange,

my late father, Donald A. Lange,

and my late niece, Rachael E. Geiger

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"I was lucky in having lived through some very exciting times and history, both big history and little history, and even having participated in a little bit of it. Those fall under the category of 'adventures'. I recommend that you get out there and have a few. They can be as simple as joining a bicycle tour or as complex as leading an expedition to Mars. Makes little difference as long as it gets your adrenaline going in a positive direction."

— Raymond E. Hampton

And out of the houses the rats came tumbling.
Great rats, small rats, lean rats, brawny rats,
Brown rats, black rats, gray rats, tawny rats.
Grave old plodders, gay young friskers,
Fathers, mothers, uncles, cousins,
Cocking tails and pricking whiskers,
Families by tens and dozens,
Brothers, sisters, husbands, wives---
Followed the Piper for their lives.

— Robert Browning
The Pied Piper of Hamelin
[1845]

The science of life....is a superb and dazzlingly lighted hall which may be reached only by passing through a long and ghastly kitchen.

— Claude Bernard
Introduction à l'Étude de la Médecine Expérimentale
[1865]

There are some things which cannot be learned quickly, and time, which is all we have, must be paid heavily for their acquiring. They are the very simplest things. And, because it takes a man's life to know them, the little new that each man gets from life is very costly and the only heritage he has to leave.

— Ernest Hemingway

You expected to be sad in the fall. Part of you died each year when the leaves fell from the trees and their branches were bare against the wind and the cold, wintry light. But you knew there would always be the spring, as you knew the river would flow again after it was frozen. When the cold rains kept on and killed the spring, it was as though a young person had died for no reason.

— Ernest Hemingway
A Movable Feast

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INTRODUCTION

The purpose of this study was to investigate the effects of androgen manipulation during prenatal and early neonatal development on the display of adult sexual behavior in female rats.

Hormonal influences on sexual behavior can be divided into two categories, organizational and activational. The organizational effects of hormones occur during stages of an organism's development. These organizational effects produce permanent structural changes in the nervous system as well as other tissues. Activational effects of hormones occur in mature animals resulting in relatively rapid and impermanent changes in structure or behavior. Both organizational and activational effects are critical components of adult sexual behavior.

Activational Effects of Hormones

Sexual behavior in the adult rat is dimorphic between males and females. Both sexes have unique actions associated with their morphology. Traditionally, the male has been viewed as the sex that initiates sex behavior, actively seeking to mount, intromit, and ejaculate with a

receptive female. The female, by comparison has been viewed primarily as reactive in her behavior, with lordosis occurring in response to male stimulation. More recently, the behavior of the female during copulation has been divided into three forms: attractiveness, perceptivity, and receptivity (Beach, 1976).

"Attractiveness" behaviors include those actions that stimulate the male. In the study of female sex behavior, little work has been undertaken to address attractiveness, as attractiveness is a response the female provides to the male but does not appear to regulate.

Proceptive behaviors, sometimes called courtship behaviors, include approach, orientation, and run-away actions in relation to the male. Proceptivity is believed to reflect the female's sexual solicitation of the male. Additional approach actions include sniffing the male, grooming the male, and run-away actions include ear wiggling, hopping and darting (Beach, 1976; Madlafousek et al., 1976, McClintock & Adler, 1978). Proceptive behaviors are especially important in mate selection by the female (McClintock, 1987), but have been commonly viewed as peripheral to actual copulation. Hence, the traditional view of female sex behavior was that it was a reactive behavior. Initial proceptive behaviors are followed by a mixture of receptive and additional proceptive behaviors.

Receptive behavior for the female includes a behavioral stance termed "lordosis" which is characterized by immobility and a stereotypical ventroflexion of the back, a lifting of the head, and a presentation of the female's vaginal opening to the male. Lordosis in the sexually receptive rat occurs in direct response to stimulation from the male. Because receptivity has been the focus of most research in the female, examination of proceptivity has lagged behind the study of receptivity.

Specific proceptive behaviors, the approach and retreat of a female from a male, were first reported in the early 1960s (Bermant, 1961; Pierce & Nutthall, 1961; Calhoun, 1962) and were called "pacing behaviors" (Bermant, 1961; Pierce & Nutthall, 1961). These pacing behaviors have been used to assess the temporal control of coital stimulation received by the female. Bermant (1961) first examined part of this pacing behavior in tests where females gained access to males through operant conditioning procedures. After a female learned to manipulate a simple lever to gain access to males, Bermant found she would press a lever at different intervals dependant upon the level of stimulation she had previously received from a male.

More recently, detailed analysis of sexual behavior in semi-natural conditions by McClintock and Anisko (1982) revealed that females exert a strong influence on the pattern of mating not previously realized in open field

arenas. McClintock's semi-natural testing arena consisted of the addition of an escape chamber that only the female could move to during copulation. In this setting, far more elaborate proceptive behavior was shown by the female than was apparent using standard laboratory methods designed to assess lordosis. The female's approach and the accompanying run-away actions were increased and dramatically altered the timing of her copulatory behavior. A predictable pattern emerged when the female was able to regulate this timing during copulation: the interval between mounts was shorter than for intromissions, and likewise the interval between intromissions was shorter than that following an ejaculation (Bermant, 1961; Pierce & Nutthall, 1961; Krieger et al., 1976; Erskine, 1985; Erskine, 1989).

Pacing is important for pregnancy induction and females allowed to pace their copulatory behavior show higher rates of pregnancy than females not allowed to pace (Gilman et al., 1979; Erskine et al., 1989; Frye & Erskine, 1990). Pacing has also been shown to enhance the effect of vagino-cervical stimulation because fewer intromissions are required in the paced copulatory paradigm to induce pseudopregnancy (Gilman et al., 1979; Erskine et al., 1989). This effect probably reflects the fact that pacing behavior facilitated prolactin surges which are important for initiation of pregnancy (Kornberg & Erskine, 1994). Pacing, in effect, appears as a behavioral mechanism that allows the

female to maximize the genital stimulation required for pregnancy. Dependance of pacing on genital sensory stimulation was further demonstrated (Bermant, 1961; Pierce & Nutthall, 1961; Krieger et al., 1976; Erskine, 1985; Erskine, 1989), when the genital area was anesthetized (Bermant & Westbrook, 1966) or the pelvic nerve was transected (Erskine, 1992). Under these conditions, pacing behavior was disrupted. Finally, pacing behavior results in changes in male/female genital contact during copulatory behavior. Females allowed to pace receive longer genital contact from a male during intromission (Erskine et al., 1989).

Sex Determination and Differentiation — Organizational Effects of Hormones

Fertilization is the initial step in a series of events leading to sexual differentiation in mammals. Penetration of the ova by the sperm sets into motion a cascade of genetic, physiological, and endocrinological processes leading to the development of a specific sex. Through fertilization, the ova receives either an "X" or a "Y" sex chromosome from the sperm to become a zygote. Sex determination results from this sex chromosome pairing of sperm and ova. If the sex chromosome pair is "XY" the genetic sex is male, whereas an "XX" pair will result in a genetic female. While the sex chromosomes establish the

genetic sex of the individual at fertilization, the primordial gonad remains bipotential during much of early development; that is, it can develop into a gonad of either sex.

Two separate teams, Ford et al. (1959) and Welshons and Russel (1959) localized the sex determining gene to the Y chromosome. In order for the primordial gonad to develop into a testis, this testis determining factor gene must be activated on the Y chromosome (Berta et al., 1990). Expression of the testis determining gene within the bipotential gonad results in production of specific proteins modulating the tissue's development into a testis. Without these proteins, the primordial gonad will differentiate into an ovary. Protein production of the testis determining gene is believed to be localized within gonadal tissue and not elsewhere in the body. More recently, a specific gene called the Sry gene has been shown to be conserved on the Y chromosome (Sinclair et al., 1990; Gubbay et al., 1991; Foster et al., 1992) and its role in human sex determination has been supported by mutation studies of XY humans that develop as physiological females (Berta et al., 1990; Jager et al., 1991; Hawkins et al., 1992a, 1992b, Hawkins 1995). Additionally, the role of the Sry gene in sex determination was supported in studies where XX mice injected with this gene showed testicular development, male secondary sex characteristics, and male mating behavior (Koopman et al.,

1991). It must be made clear that additional genes are necessary and were lacking to induce fertility in these mice (Burgoyne et al., 1992; Conway et al., 1994) because all were sterile. It is believed that genes have a very localized effect on tissue determination. The idea of a localized effect of the gene responsible for testis determination is supported by cases of differentiation into one testis and one ovary within the same individual (van Niekerk, 1976). In such an instance, the gonad with gene expression develops into a testis and the other gonad, lacking the expression of the gene becomes an ovary.

After induction of testicular development, the gonad of a mouse condenses into a distinct, identifiable tissue between day 10 and 11 following fertilization (Capel & Lovell-Badge, 1993). Once the gonad has differentiated into either an ovary or a testis, the hormones produced by the gonad direct whether the individual develops anatomical structures of a male or a female. The first mammalian hormone identifiable as an indication of testis development is produced by Sertoli cells and is called Anti-Mullerian Hormone (also referred to as Mullerian Inhibiting Substance) (Cate et al., 1986; Picard & Josso, 1984; Musterberg & Lovell-Badge, 1991). Through first the action of the gene, then action of the gonadal hormones, a pattern of tiered development is seen. The process of sex differentiation can

be summarized as: genetic sex determines gonadal sex which in turn determines phenotypic sex (Jost, 1979).

If a testis develops, androgens from that gonad induce development of masculine peripheral tissues and masculine organization of the brain, which provides the substrate for development of masculine sex behavior. Additionally, androgenization by Leydig cells for virilization of genitalia and Sertoli cell produced AMH cause regression of Mullerian ducts (Picard & Josso, 1984).

If an ovary develops, estrogens are produced, albeit in much smaller quantities than the androgens produced by the testis (Dohler et al., 1982). Estrogen production by the ovary is so low that earlier researchers believed the embryological state for female development was ahormonal: a female phenotype was believed to develop in the absence of gonadal hormone stimulation. However, mammalian female sex chromosome disorders (such as Turner's Syndrome in humans) appear to prevent normal development of the ovaries leading to a lack of production of estrogen. Prenatally this lack of estrogen is associated with incomplete feminization of peripheral anatomy and a lack of sexual maturity in adulthood (Zinn et al., 1993). This lack of prenatal estrogen may also result in incomplete feminization of the brain prenatally (Dohler et al., 1982). Therefore, complete sex differentiation of both sexes may rely on gonadal hormones secreted by the respective differentiated gonad.

Sex Differentiation – Origins of Hormonal Effect

In the earliest recorded study of endocrinology, adult sex morphology and behavior were examined in chickens (Berthold, 1849). Castration of young male chicks was shown to inhibit adult sexual characteristics such as comb development, wattle maturation, and male-typical vocalizations. Berthold postulated that the testes secreted a form of blood "conditioner" which altered the blood to facilitate development of male characters, yet the actual mechanism remained unclear on how these secretions caused changes in the adult. It was not until 1935 that this testicular "conditioner" was synthesized by Ruzicka and Wettstein and named testosterone (Hadley, 1996). In a similar vein, adult sexual behavior in mammals relies heavily on the gonadal hormone state of the animal. For the Norway rat (*Rattus norvegicus*), behaviorally relevant gonadal hormone action occurs prenatally, neonatally and in adulthood. In an early study of rodents, transplants of gonadal tissue between the sexes early in neonatal life (before the 10th day after birth) disrupted normal secretion patterns. When a female received a transplant of male gonadal tissue in early neonatal life, her estrous cycles were disrupted and she was acyclic in adulthood (Pfeiffer, 1936). In the reverse situation, neonatally castrated males showed cyclic patterns of secretion of luteinizing hormone from the pituitary similar to the pattern in normal adult

females (Pfeiffer, 1936). Cyclic patterns of hormone secretion were first found to be under neural control in rabbits when Harris (1937) induced ovulation via electrical stimulation in regions of the hypothalamus. It was suggested that differential exposure to androgens during neonatal life resulted in sex differences in the control centers of the hypothalamus in adulthood (Harris, 1955). Differential organization of hypothalamic control centers occurs very shortly after birth, leading to females showing cyclic patterns of gonadal hormone secretion and males showing tonic patterns of gonadal hormone secretion.

Research in the laboratory of William C. Young in the 1950s first linked adult sex behavior to earlier development. In a landmark paper, Phoenix, Goy, Gerall, and Young (1959) reported that the endocrinological organization of adult sex behavior is due to the pattern of hormonal exposure during early development — just as primary and secondary sex characteristics of body morphology are. Female guinea pigs exposed to androgens during prenatal development showed a dramatic decrease in lordosis response when tested at maturity. In addition, many of these females showed male-like mounting behaviors. Based upon these findings, the authors proposed the "organizational hypothesis" of sexually dimorphic behaviors. This hypothesis states: androgens secreted during prenatal development organize the components of the nervous system

utilized in adult sex behavior. The suggestion of perinatal testosterone exposure affecting nervous system development was very novel for it was argued that the effects of perinatal hormones were on genital morphology and morphological changes to the genitals were the way in which behavior became modified. However, later work has shown several central nervous system structures important for sexual behavior are dimorphic. In the sexually dimorphic nucleus of the preoptic area of the brain (SDN-POA), the volume of this region is 3-8 times larger in males than in females (Gorski et al., 1978). This dimorphic difference in volume between males and females has been shown to depend on testosterone exposure around birth (Jacobson et al., 1981; Simerly et al., 1985) and the critical period of exposure to testosterone has been found to be anywhere from prenatal day 18 to postnatal day 5 (Rhees et al., 1990a; Rhees et al., 1990b).

The organizational hypothesis offered a strong argument for relating the effects of testicular androgen secretion on behavior to the organization of the brain prenatally. However, one confounding variable was found when it was shown that estrogen exposure would also defeminize the female, just as occurred with androgen exposure (Feder & Whalen, 1965).

Effects of Estrogen and Testosterone

Replacement of estrogen alone in ovariectomized, adult female rats facilitated lordosis but did not promote proceptive behaviors (Hardy & DeBold, 1971; Whalen, 1974). However, proceptive behaviors were shown when both estrogen and progesterone were given (Tennent et al., 1980; Erskine, 1985). When pacing was examined, the combined estrogen and progesterone replacement resulted in standard pacing behaviors (Erskine, 1985). In these studies estrogen alone was equally effective in promoting pacing behavior (Gillman & Hitt, 1978). Induction of pacing with estrogen alone may have been a result of the method of measuring pacing in the Gillman and Hitt study where elevated platforms were used to assess pacing instead of the more common divided arena.

Exposure of hamster females to estrogen postnatally was found to also abolish lordosis in adulthood. Androgens aromatized into estrogens in the brain via the aromatase enzyme were found to play a significant role in masculinization and/or defeminization during development of both sexes (Paup et al., 1972; Coniglio et al., 1973).

Aromatase is an enzyme that promotes the conversion of testosterone to estrogen, and has been localized in hypothalamic brain regions suspected of governing sex behavior (Reddy et al., 1974; Naftolin et al., 1975). Through radioactive labeling of testosterone injected into

the rat, it was shown that testosterone is converted into estrogen in the hypothalamus (McEwen & Krey, 1984).

Because females can exhibit mounting behavior (Sodersten, 1972) and males can exhibit lordosis (van de Poll & van Dis, 1977), the sex differences in female and male sexual behaviors must be considered as relative instead of absolute. This relative nature suggests that females capable of showing both masculine and feminine behavior are influenced not only by her own gonadal hormones, but also by testosterone the female is exposed to during development. Hence, testosterone likely plays a significant role in the development of adult female sex behavior.

As support of the idea for the influence of testosterone in the suppression of lordosis behavior, normal female sex behavior can be altered by manipulation of perinatal testosterone levels. High levels of mounts, intromissions and even occasional ejaculation-like behaviors are seen in females perinatally treated with testosterone (Sachs & Thomas, 1985). This conforms to the expectation of the higher levels of androgen in the circulation in males than in females perinatally (Pang et al., 1979; Slob et al., 1980). Males begin producing testosterone via the testis around prenatal day 14 and a large surge in testosterone in seen on prenatal days 18 and 19. This surge is critical for behavioral masculinization of males (Weisz & Ward, 1984). However, testosterone also partially defeminizes and

suppresses the development of female behaviors. Females perinatally treated with testosterone show lower receptivity and proceptivity in adulthood (Whalen & Edwards, 1967). Partial defeminization is also seen in females prenatally exposed to testosterone (Gladue & Clemens, 1978).

Naturally occurring prenatal androgen exposure takes place in some females due to their intra-uterine position. If a female develops between two male siblings, androgens from the neighboring males will enter the circulation of the female (Clemens, 1974; Clemens et al., 1978). These androgens from surrounding males can be aromatized into estrogens and will increase the behavioral masculinization and defeminization of the female (Coniglio & Clemens, 1976; Coniglio et al., 1973), suggesting a central action for the effect of aromatizable androgen in organizing behavior. These studies on the role of aromatizable androgens developed into a complementary concept to the organizational hypothesis named the "aromatization hypothesis" to address the findings of Feder and Whalen in which early estrogen exposure was also shown to masculinize females. Early estrogen treatment was shown to masculinize ovulation and diminish lordosis. The aromatization hypothesis suggests that estrogen aromatized from testosterone in early development will masculinize the brain of developing foetuses.

An implication of the aromatization hypothesis is that maternally produced estrogens might also induce masculine brain organization in female pups. However, masculinization due to maternal estrogens may be prevented by a protein present during prenatal and perinatal development called alpha-fetoprotein (AFP) (Raynaud et al., 1971). AFP selectively binds to estrogen to regulate circulating maternal estrogens within the foetus (McEwen, 1978). This regulation is believed to limit the female's exposure to circulating estrogens (see Figure 1) (O'Malley, 1974), but low levels of circulating estrogen may play a crucial role in normal female brain development (Dohler et al., 1982; Toran-Allerand, 1986; Witcher & Clemens, 1987).

While the inclusion of neonatal differentiation as a factor in adult sex behavior was begun by Phoenix et al. (1959) and further developed by Barraclough and Gorski (1962), it was Harris (Harris, 1964; Harris & Levine, 1965) who suggested that the effects of gonadal hormone secretion may extend into prenatal life. A developmental time line for the rat (Figure 2) illustrates a number of significant events during prenatal development. The total number of days between fertilization and adult maturity in the female rat is approximately 70 days. The 21 days of gestation represents 30% of the entire time period available for maturation. Gonadal bipotentiality is lost near day 10 of gestation, and at that time, development into a testis

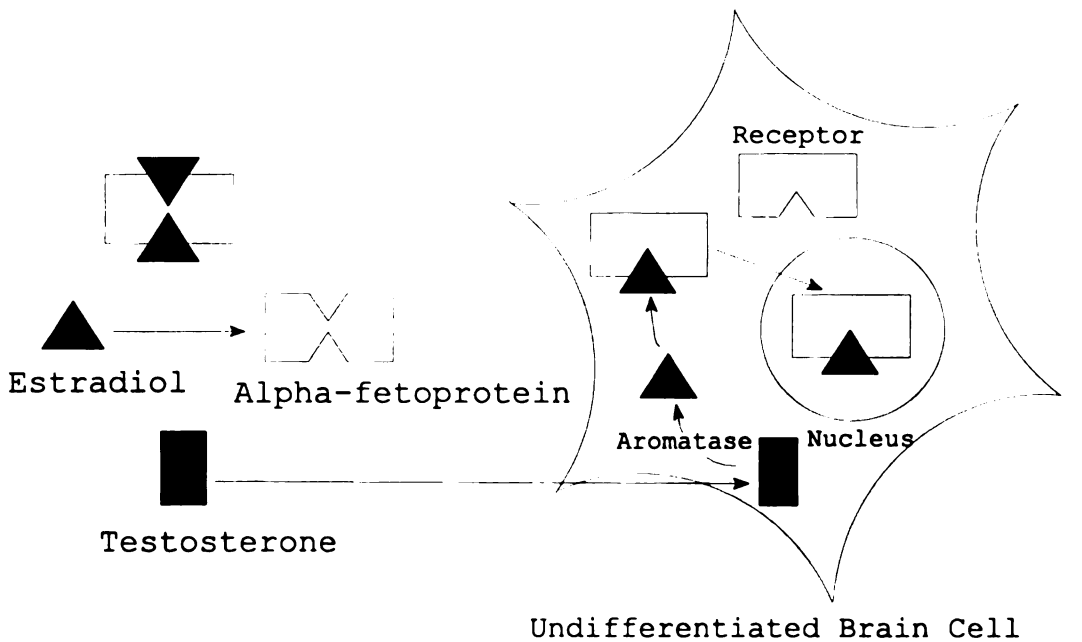

Figure 1. Alpha-fetoprotein serves to prevent estradiol from entering undifferentiated brain cells. Testosterone does not bind with alpha-fetoprotein and is free to enter the bipotential brain cell, become aromatized to estradiol and bind to a cytoplasmic estradiol receptor. Once bound to the receptor it enters the nucleus and can lead to masculinization or defeminization of the cell.

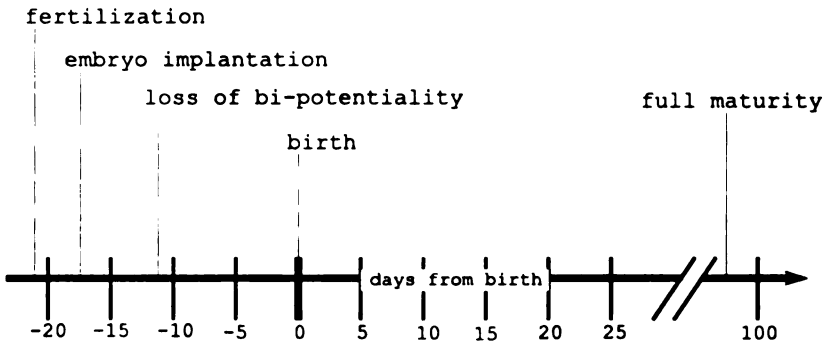

Figure 2. Rat Developmental Time Line Showing Key Developmental Events.

begins if the Sry factor is present (Berta et al., 1990). In the male, there is also a rise in testosterone levels from the testis during prenatal days 14-18 in the rat (Picon, 1976) that helps to organize body morphology and brain development. From day 18 until parturition, levels of testosterone in the male declines.

Hence, the female in development is exposed to both estrogens and androgens during development and these may play a critical role in the organization of adult female sex behavior.

Aromatization of Testosterone — Effects on the Neonate

The seemingly contradictory idea of both testosterone and estrogen playing a similar role in the development of sex behavior was not initially clarified through studies of development, but instead was uncovered in studies of hormonal changes in the adult brain. Beyer et al. (1969) found that aromatization of testosterone into estrogen occurred in adults. Only later was support found for the aromatization of testosterone during development for both males and females (Paup et al., 1972; Coniglio et al., 1973. Further research localized active sites of the aromatase enzyme in the hypothalamus (Reddy et al., 1974; Naftolin et al., 1975). Radioactive labeling proved aromatization of testosterone into estrogen does occur within regions of the hypothalamus (McEwen & Krey, 1984).

Aromatization — The Conversion of Testosterone into Estradiol

Testosterone can act in one of three ways. It can act directly as testosterone, it can act indirectly by being converted into one of several non-aromatizable androgens, or it may act indirectly by being aromatized into estradiol (Figure 3). Masculinizing effects for testosterone have been suggested (Paup et al., 1972; Clemens, et al., 1978; Gladue et al., 1978; Weisz & Ward, 1984), but the actual metabolite(s) responsible for this masculinization was unresolved.

Evidence, however, points to a role for testosterone aromatized into estradiol as being critical for defeminization and masculinization of the nervous system which directs adult sex behavior in rats. Because the neonatal brain of both sexes in rats has the aromatase enzyme (Reddy et al., 1974) and because non-aromatizable androgens are much less effective in defeminization (Paup et al., 1972; Coniglio et al., 1973) than are aromatizable androgens it is suggested that this mechanism is directed by estrogenic activity. This is further supported by the diminished effect of testosterone shown in neonate females exposed to anti-estrogens (McDonald & Doughty, 1972).

Androstatriene-3,17-dione (ATD) is a steroidal compound that inhibits activity of the aromatase enzyme. ATD prevents the aromatization of testosterone into estrogen

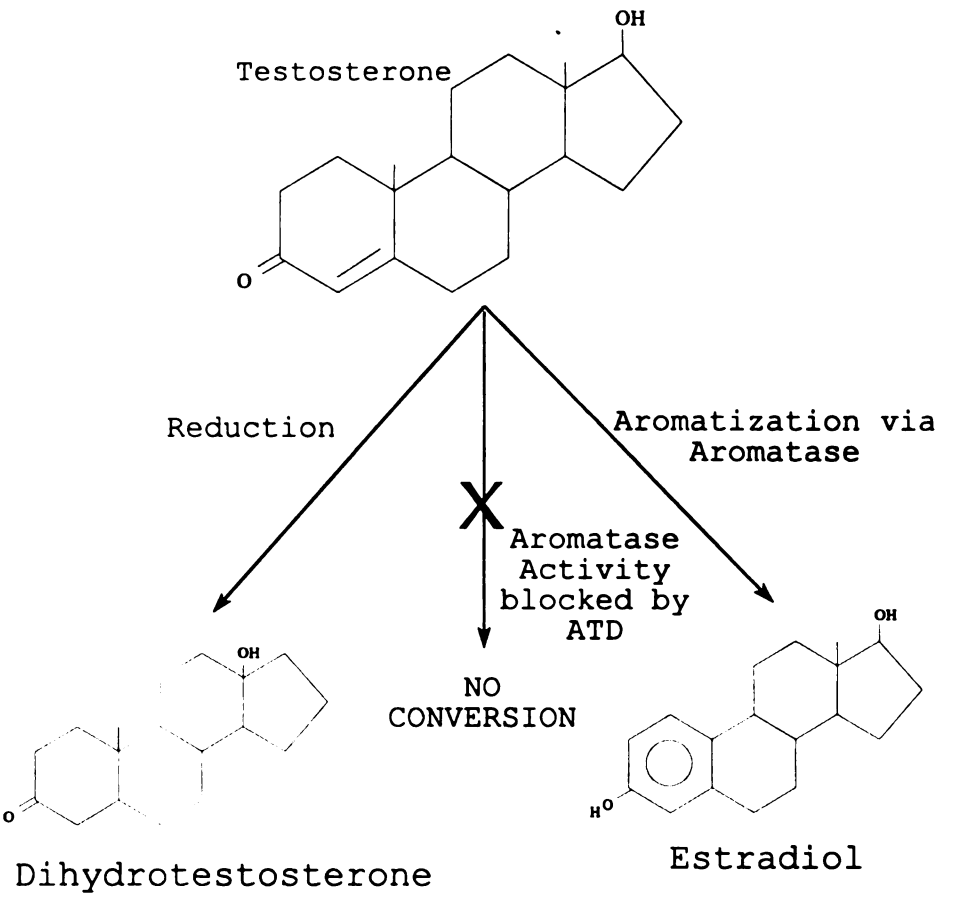

Figure 3. Illustration of reduction and aromatization of testosterone to dihydrotestosterone and estradiol and effects of ATD on aromatase.

(Brodie et al., 1976; Brand et al., 1991). In the adult, ATD blocks mounting behavior in adult male rats (Christensen & Clemens, 1975; Sodersten et al., 1986) and neonatal exposure to ATD inhibits defeminization in a similar manner as castration surgery in males (Vreeburg et al., 1972; McEwen et al., 1977; Brand et al., 1991). Because ATD has proven useful in inhibiting activity of the aromatase enzyme, it was selected for use in the manipulation of testosterone and related metabolites in the new research presented in this document.

In female rats, adult exposure to ATD blocks lordosis behavior (Brodie et al., 1976) and blocks testosterone induced mounting behavior in female guinea pigs (Roy & Goy, 1988), whereas prenatal ATD exposure has resulted in both sexes showing enhanced lordosis in adulthood (Clemens & Gladue, 1978). These findings suggested that ATD diminished estrogen levels necessary to limit the feminine organization of behavior. Prenatal ATD exposure resulted in increased feminine sexual behavior in adulthood (Gladue & Clemens, 1980), but feminization can be reversed in the neonatal rat, for blockage of the aromatization of testosterone defeminized rats (Booth, 1978). The manner in which female fetuses are believed to block masculinization of the nervous system via aromatization like seen in males is through the serum protein alpha-fetoprotein which binds to estrogen to

prevent it from entering the undifferentiated brain cells of females (Raynaud et al., 1971).

Comparison — Activational and Organizational Effects

In adulthood, hormones activate specific sexual behaviors that have been organized by perinatal and prenatal hormones. The display of feminine sexual behavior in the rat is controlled by the different concentrations of hormones present resulting in a cyclic pattern of behavior associated with the stage of ovulation (Morali & Beyer, 1979).

One early measure that helps illustrate the organizational effect of hormonal concentrations during development is seen in the variation in anogenital distance statistics. Females prenatally exposed to androgens show larger anogenital distances at birth, indicating masculinization of these androgen sensitive tissues. Behaviorally this is associated with an increased level of mounting behavior in adulthood (Clemens et al., 1978). It is known that variation in androgen exposure naturally occurs for females *in utero* (Pang et al., 1979; Slob et al., 1980), and these endogenous androgens are suspected of arising from either the placenta (Chan & Leathem, 1975; Gibori & Sridaran, 1981; Vreeburg et al., 1983) or from males developing within the same uterine horn (Clemens & Coniglio, 1971). Variation in morphology and behavior of

female rats due to intra-uterine position during development follows a pattern consistent with increases in androgen exposure. Namely, females nearer to males within the uterine horn exhibit more masculine anogenital distances and adult sex behavior patterns (Clemens et al., 1978). Similar masculinization of females near males *in utero* has been seen in gerbils (Clark & Galeff, 1988).

Therefore, while there is currently debate as to the sources of androgens females are exposed to in prenatal development, it is known that females do have exposure to androgens. Hence, this dissertation presents experiments that examine the effects of hormone exposure within the prenatal environment on aspects of sexual differentiation in female rats. Specific emphasis was given to the effects testosterone and metabolites derived from testosterone have on the female's proceptive behavior expressed during her pacing of copulation with a male. Studies on the effects of androgens on adult female sexual behavior have thus far only examined the role of androgens on the development of female's lordosis posture. This dissertation presents novel work to help clarify the role prenatal hormones have on the organization of adult female's timing during copulation. This timing of sexual behavior is critical, for the pattern, timing, and quantity of coital stimulation received by the female exerts strong effects on her reproductive success.

MATERIALS AND METHODS

Animals

Long-Evans rats were used in all experiments. For each experiment, maternal females were obtained from Harlan-Sprague Dawley Incorporated, P.O. Box 29176, Indianapolis, IN 46229. Two groups of service males were also obtained from Harlan-Sprague Dawley Inc. The initial group of males was used for mating to produce the experimental and control animals used in this study. A second group of males was obtained three weeks prior to the start of behavioral testing. These latter males were purchased in one group to create consistency in age and origin of the service males in the behavioral tests. All male rats were purchased at 90 days of age.

Breeding females were purchased from Harlan-Sprague Dawley Inc. at 60 days of age and were initially housed in groups of three in cages 50cm x 25cm x 20cm. After mating, females were housed singly. Males were housed in groups of three in plastic cages of the same size as those for the females.

Offspring from impregnated females were housed as complete litters until weaning at day 30. After weaning, the

animals were group housed according to sex and treatment until day 60, when only the females were kept and housed in groups of 3 to 5 through the course of the experiment. All animals were maintained in a reverse dark-light cycle (dark phase from 11am until 9pm (10 hour phase) and light phase from 9pm until 11am (14 hour phase)).

Treatments and Procedures

Below are outlined the general treatment groups for all experiments. More detailed information is provided in the specific methods for each experiment.

Breeding females were randomly assigned to one of the following treatment groups: a) a testosterone propionate group (TP) that received daily injections of TP in a sesame oil vehicle, b) a control group for the TP group that received only the oil vehicle (TP control), c) an androsterone group (AND) that received injections of androsterone benzoate in a sesame oil vehicle, d) a testosterone benzoate group (TB) receiving TB in a sesame oil vehicle, e) a benzoate group receiving benzoate dissolved in sesame oil (B), f) another control group (for the groups in "c" and "d") that received only the sesame oil vehicle (control), g) a group receiving the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD) via silastic capsules, and finally, h) a control group to the ATD which received blank silastic capsules (ATD control). In the

reporting of results, the animals in the benzoate group (e) and the oil control group (f) were combined (B-control).

The date of mating was considered day 0 of gestation. In the TP & TP control and the AND & TB & B-control groups, mothers of each litter of animals were given injections daily from day ten of gestation through parturition.

All pregnant females in the ATD & ATD control study were surgically implanted with silastic capsules on day 10 of gestation. Surgery involved anesthetizing the animal with pentobarbital and making a small incision at the nape of the neck. A total of 120mm of ATD filled silastic capsules were implanted subcutaneously at this location in the experimental group. The ATD control group underwent identical surgical procedures except that each received 120mm of empty silastic capsule. The silastics in this experiment were kept in place in the mother of the litters from day 10 of gestation through parturition.

Day 10 of gestation was chosen for the start of both the prenatal injections and the capsule implants in the mothers because it allowed for implantation of the embryo but is prior to loss of bi-potentiality. Treatments were also chosen for this time period to mimic the naturally occurring times of prenatal androgen secretion by the males. None of the various treatments of the mothers or pups interfered with parturition, lactation or parental behaviors of the mothers.

Pups from all litters were sexed at birth (day one postnatal life), weighed and the ano-genital distance was recorded. The ano-genital distance is the distance between the center of the anal opening and the genital protuberance. Measurements were taken using metric Vernier calipers. After recording the weight and ano-genital distance measurements, all pups were toe tagged for future identification.

Surgical Procedures

Two surgical techniques were used for each experimental animal. The first, toe clipping, occurred on the day of birth. Each animal was given unique identification via amputation of a specific number and location of toes. The identification of neonatal animals through toe tagging is a standard procedure that has no known effect on maturation or adult sex behavior.

The second surgical procedure in the test animals was ovariectomy. Animals in the ATD/ATD control and TP/TP control groups were ovariectomized between day 100 and 105. Females in all other groups were ovariectomized at day 55. Ovariectomy was performed using pentobarbital anesthesia (50mg/kg body weight); incisions were made using a bilateral flank approach to remove both ovaries. Four days after surgery, females were started on a cycle of hormone replacement consisting of three consecutive days of estrogen

followed by one day of progesterone each week throughout the experiment.

Animals were weaned from the mother at postnatal day 30 and their sex was re-confirmed along with their toe number. If a juvenile toe tag was not discernable from those expected, the animal was dropped from the study.

Behavioral Testing and Adult Hormone Treatments

Feminine Sexual Behavior – General

Beginning four days after ovariectomy, females received exogenous estrogen and progesterone treatment via injection for four days (three days of estrogen followed by one day of progesterone). This protocol started a weekly hormone regime maintained throughout the experiment. Once per week, during the two weeks post-surgery, females were exposed to sexually vigorous males on the day of the progesterone injection in both a traditional copulatory chamber and in a pacing arena. One week after these two "pre-tests," recorded tests of the female's sexual behaviors began on a weekly basis.

Feminine Sexual Behavior – Lordosis

Tests for lordosis response were made using the traditional testing situation. For each test, a single, sexually vigorous stud male was placed in a Plexiglass® observation chamber (50cm x 50cm x 50cm) for a period of 10

minutes to acclimate to the test situation. Following acclimation, the hormonally primed test female was placed with the male. The frequency of lordosis responses of the female to the male's first 10 full mounts or intromissions were recorded. A full mount was defined as a mount that included flank palpations of the female and pelvic thrusting by the male. Lordosis was defined as a deep arching of the back with elevation of the head and perineum (Hardy & DeBold, 1971). Lordosis scores were rated using a 4 point numerical system (scores from 0 through 3). A score of "0" indicated no lordosis. A score of "1" indicated a slight arching of the back to the stimulation from the male. A score of "2" is considerably more pronounced a form of lordosis and was often accompanied by intromission by the male. A score of "3" indicated a full lordotic response. In scores of "2" and "3" there is a clear flexion of the female's back and at least a small tilting of the head. In a lordosis score of "3," the arching of the back was extremely pronounced as was the tilt of the head and both often appeared exaggerated.

Response frequencies were used to calculate a "lordosis quotient" (LQ) for each animal. LQ is defined as the total number of lordosis responses with an intensity of 2 or 3 divided by the total number of mounts with the quantity multiplied by 100 (standard practice is to use 10 as the total number of mounts observed).

Feminine Sexual Behavior – Pacing

Tests for female pacing were conducted using a modification of methods employed by Erskine (1985). The observation chamber (a Plexiglass® chamber with an overall size of 50cm x 50cm x 50cm as in the lordosis test) was divided into two arenas by a Plexiglas® barrier. The female-only chamber (also called the "escape chamber") was 15cm x 50cm x 50cm, and the chamber containing the male used during copulation was 35cm x 50cm x 50cm. The dividing wall between the two chambers had a series of 4 square holes (4cm x 4cm) along the bottom through which the female could pass but the male could not. The male was placed in the larger chamber for 10 minutes prior to testing to allow for acclimation. After acclimation, a female was placed in the escape chamber to start the paced copulatory test. Behavioral measurements of the female were recorded using one of two behavior recording programs written for use on PC/DOS based systems (Karu, 1995; Rakerd et al., 1988). Measurements included frequencies and time intervals between mounts, intromissions, and ejaculations received by the female. Additionally, the latency of the female's initial approach to the male was recorded.

Hormonal Treatments during the Testing Regimes

The hormonal regime given to each test female prior to testing consisted of single daily injections of estradiol

benzoate in a sesame oil vehicle (each dosage = 0.5ug) at 72, 48, and 24 hours preceding the copulatory tests. Additionally, four hours prior to the start of testing, a single injection of progesterone (dosage = 0.5mg) in a sesame oil vehicle was given.

Solutions

ATD used in this experiment was administered through silastic capsules. Medical grade silastic tubing (1.47mm ID/1.96mm OD) was sealed at one end with silicone sealant (Medical Grade Dow Corning Silicone) and then filled to a length of 20mm with ATD. After filling, the remaining open end was closed with silicone sealant and allowed to air dry for at least one week prior to use. Empty silastic tubes were used for the control group. Prior to implantation, the silastic capsules were placed in a 100% ethanol solution (10ml/capsule) for one hour. This fluid was then drained and the silastic capsules were soaked in a 1% bovine serum albumin solution for 24 hours (10ml/capsule) to activate the capsule.

Steroidal solutions (testosterone propionate, testosterone benzoate and androsterone benzoate) were made by dissolving crystals of steroid in a sesame oil vehicle. Full dissolution was assured by placing each container of steroids in a warm (45 degree Celsius) oven for 12 hours. This procedure was used in the making of the benzoate

control solution. The oil control vehicles were also placed in the oven for 12 hours.

EXPERIMENT #1: PRENATAL ANDROGEN EXPOSURE INFLUENCES THE ORGANIZATION OF FEMALE PACED SEXUAL BEHAVIOR

Introduction

Postnatal exposure to testosterone will defeminize female rats (Ward, 1974; Whalen, 1974). However, the consequences of prenatal exogenous androgens on a female's timing of copulatory behavior with the male are largely unexplored. If prenatal androgens were to exert similar effects as postnatal androgen in defeminizing females, the prenatal exposure would be expected to lead to defeminization of these timing behaviors. Hence, it was hypothesized that the timing of the intervals of copulatory behavior would decrease in females exposed prenatally to androgens. With the use of testosterone, this experiment will broadly examine the spectrum of the action of androgens and estrogens (through aromatization of testosterone) on the timing of sexual behavior in the female.

Method

Pregnant females were injected intramuscularly with 0.5mg of testosterone propionate daily from day 10 to parturition.

RESULTS

Effects of Prenatal Testosterone Treatment on Female's Pacing of Copulatory Behavior

Testosterone exposure during early prenatal development led to alteration of the female's pacing behavior. Testosterone-exposed females showed an increase in their intromission rate when compared to controls (Figure 4) (t-test, $p<0.05$). This increased rate of intromissions did not significantly decrease ejaculation latency. Prenatal treatment with testosterone also abolished the post-ejaculatory refractory period (PERP) in a majority of females. PERP is the interval following an ejaculation where a female occupies the non-copulatory chamber. Because many females did not show a PERP, a significant difference is seen between control and treatment groups (Figure 5) (Chi-Square, $p<0.05$) when the presence or absence of PERP is examined.

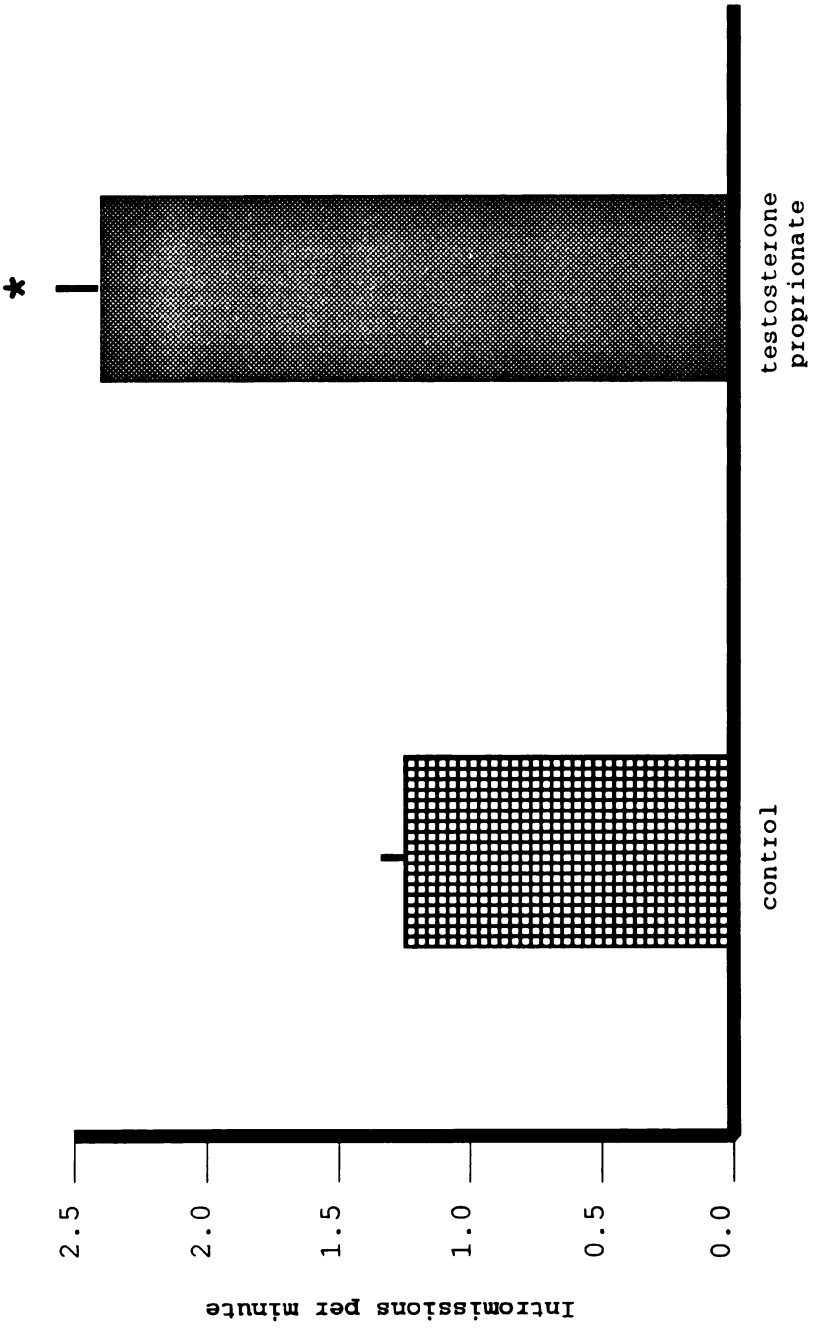

Figure 4. Comparison of adult intromission rates in control and testosterone proprionate (TP) treated females (t-test, $p<0.05$).

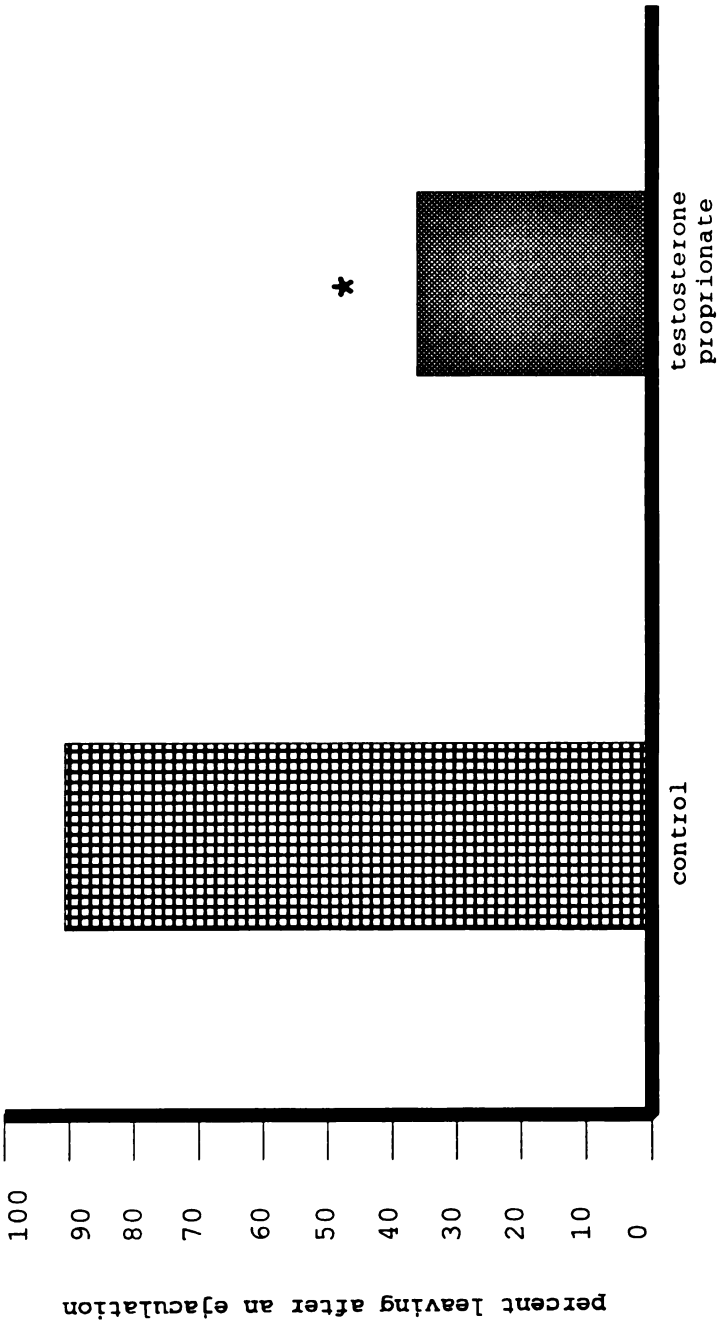

Figure 5. Comparison of refractory periods in control and testosterone proprionate (TP) treated females after receiving one ejaculation (Chi-Square, $p<0.05$).

EXPERIMENT 2: PRENATAL INFLUENCES OF TESTOSTERONE BENZOATE AND ANDROSTERONE BENZOATE ON FEMALE SEXUAL BEHAVIOR AND GENITAL MORPHOLOGY

Introduction

To further elucidate the role of prenatal exposure to androgens on the development of the timing of sexual behavior in the adult female rat, analysis of the effects of one of the reduced metabolites of testosterone was examined. Androsterone, a reduced metabolite of testosterone, has been shown to be present at high levels in developing foetuses of both sexes (Slob & Vreeburg, 1985). Androsterone is a non-aromatizable androgen, and hence the use of this compound circumvents the possible estrogenic effect of aromatized testosterone on female pacing behavior. Testosterone given neonatally has been shown to decrease the lordosis quotient (Clemens et al., 1970). Additionally, when given prenatally, flutamide (an anti-androgen) results in a significant increase in the lordosis quotient in females at adulthood (Gladue, 1979). This use of flutamide suggests that androgens will likely decrease the lordosis quotient if given prenatally, but direct evidence is needed. Because of this, a testosterone group was added to broaden the scope of this experiment to examine the effects of prenatal exposure

to non-aromatizable androgens (in this case androsterone benzoate) or aromatizable androgens (in this case testosterone benzoate) on lordosis quotient as well as the primary goal of examining the effects of these compounds on the timing of adult sexual behavior in females. This study, therefore, examines the effects of prenatal administration of exogenous androsterone benzoate on female adult sex behavior and additionally to examine the effects of exogenous prenatal testosterone benzoate on female adult sex behavior.

Methods

Pregnant females were intramuscularly injected with androsterone benzoate or testosterone benzoate dissolved in a sesame oil vehicle from day 10 to parturition.

Because the benzoate form of androsterone was selected as the exogenous metabolite, a testosterone group was added using the benzoate form of testosterone to allow direct comparison between the role of testosterone and androsterone. To control for this different binding molecule from experiment 1, a vehicle control, and a binding molecule control (benzoate in oil) were included in this study. In the results, the benzoate and vehicle control groups were combined into one large sample.

RESULTS

Effects of Prenatal Androsterone Treatment on Lordosis in Females in the Non-Paced Environment

No significant differences were noted between the lordosis quotients of females exposed to androsterone benzoate, testosterone benzoate, or the oil and benzoate groups. All females exhibited high lordosis quotients (Figure 6) (t-test, p=not significant). When lordosis intensity was examined, androsterone benzoate exposed females showed a significant decrease in the intensity of their lordosis posture (Figure 7) (t-test, $p<0.05$).

Effects of Prenatal Androsterone Treatment on the Genital Morphology of Females

Significant virilization was seen in both the androsterone benzoate and testosterone benzoate treatment groups in terms of the anogenital morphology of the offspring (Figure 8) (t-test, $p<0.05$).

Effects of Prenatal Androsterone Treatment on Female's Pacing of Copulatory Behavior

Androsterone benzoate exposure during prenatal development led to alterations of the female's pacing of sex

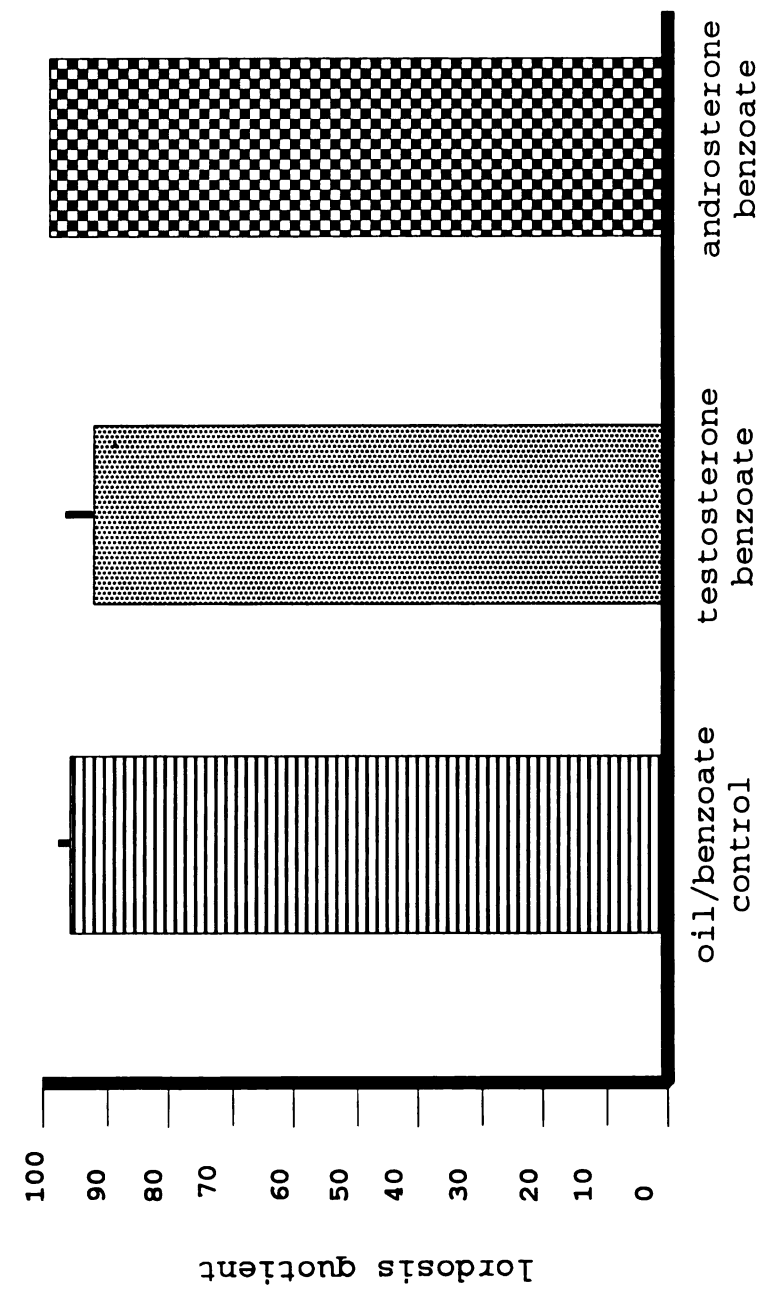

Figure 6. Comparison of adult lordosis quotients in oil/benzoate control, testosterone benzoate, and androsterone benzoate treated females (t-test, p=not significant).

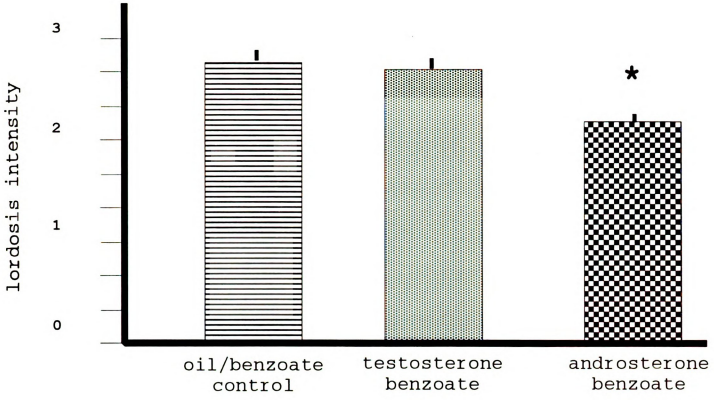

Figure 7. Comparison of adult lordosis intensity in oil/benzoate control, testosterone benzoate, and androsterone benzoate treated females (t-test, $p<0.05$).

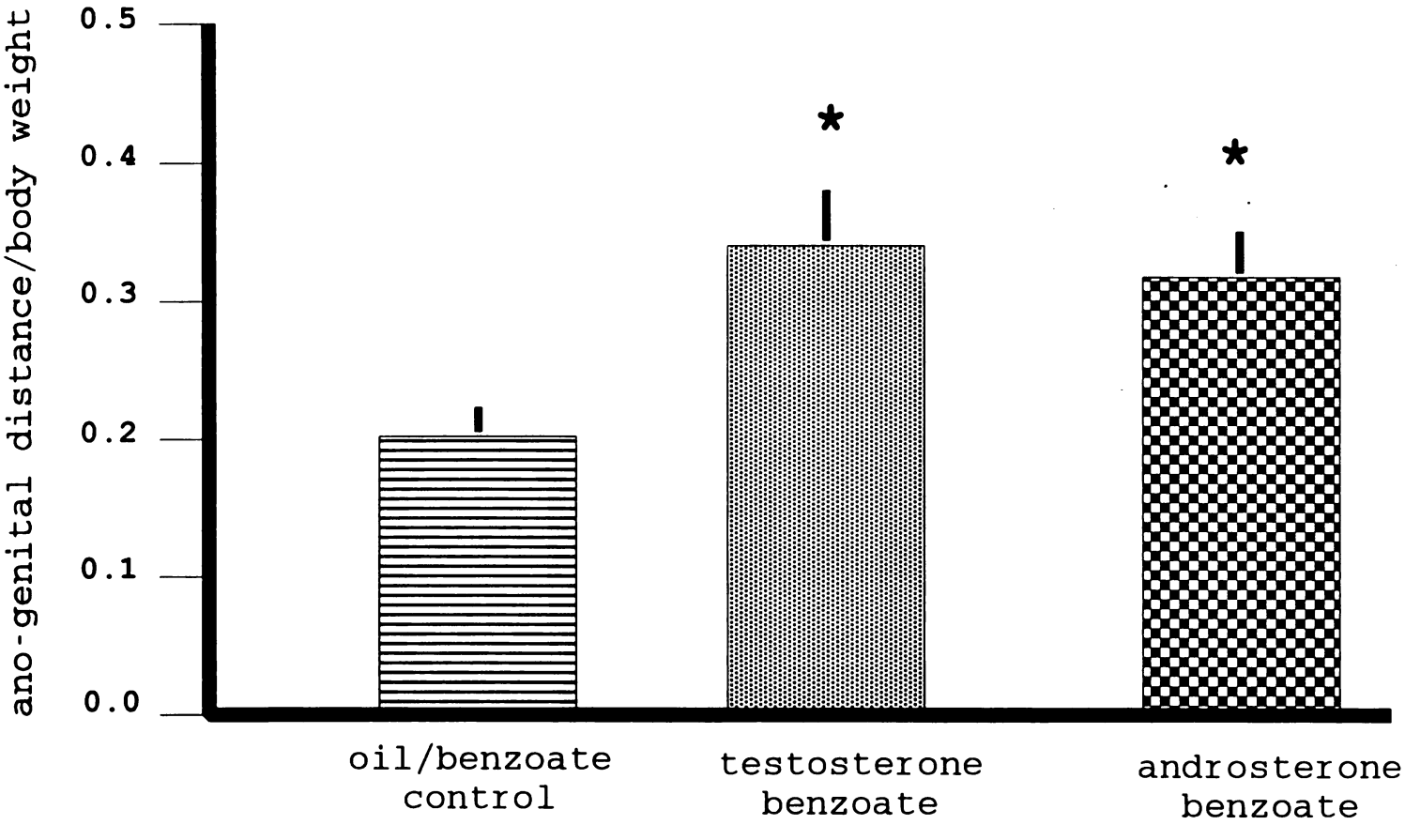

Figure 8. Comparison of neonatal ano-genital distance/body weight statistics in oil/benzoate control, testosterone benzoate, and androsterone benzoate treated females (t-test, $p<0.05$).

behavior as was seen in experiment #1. The intromission rate of animals exposed to androsterone benzoate was increased when compared to controls (Figure 9) (t-test, $p<0.05$). Prenatal treatment with either androsterone benzoate or testosterone benzoate abolished the post-ejaculatory refractory period in a significant majority of exposed females. This abolishment is shown in a comparison of females displaying a PERP in control and androsterone exposed treatments (Figure 10) (Chi-Square, $p<0.05$).

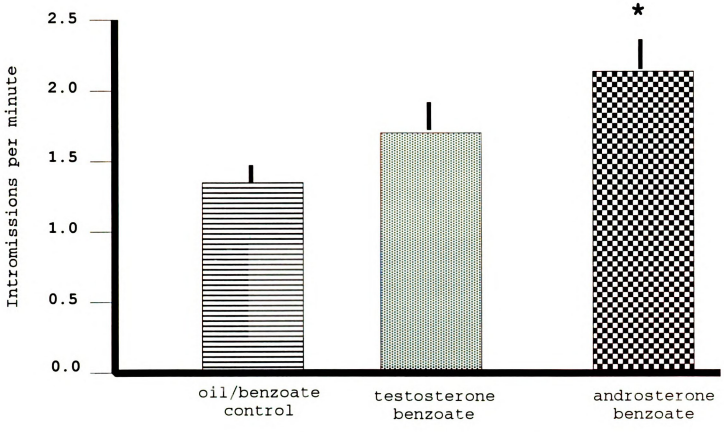

Figure 9. Comparison of adult intromission rates between oil/benzoate control, testosterone benzoate, and androsterone benzoate treated females (t-test, $p<0.05$).

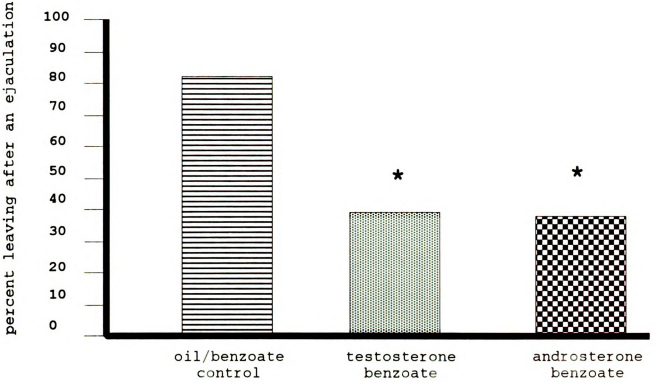

Figure 10. Comparison of adult refractory periods in oil/benzoate control, testosterone benzoate, and androsterone benzoate treated females (Chi-Square, $p<0.05$).

EXPERIMENT 3: PRENATAL INFLUENCES OF ATD ON FEMALE SEXUAL BEHAVIOR AND GENITAL MORPHOLOGY

Introduction

ATD is used to decrease circulating levels of estrogens (Reddy et al., 1974). Androgens play a significant role in the organization of sexual behavior in the adult female, but it is possible for the effects of the androgen, testosterone, to arise from the aromatization of testosterone into estrogen. Experiment #2 examined the effects of both aromatizable and non-aromatizable androgens. In this experiment the goal is to further limit the foeti's exposure to estrogen and hence further clarify whether the alteration of the timing of paced sexual behavior in the adult female is wholly an androgenic action in prenatal development or if there is also an estrogenic component to the development of this behavior. Using naturally circulating androgens, it is anticipated that ATD exposure will limit conversion of aromatizable androgens into estrogens in circulation. A possible risk with this protocol is that the limiting of the aromatization pathway may lead to higher circulating levels of endogenous androgens, but the levels should be at far lower levels than

were present in the exogenous exposures to androgens used in experiments #1 and #2.

As a first step to understanding the possible contribution of estrogens aromatized from testosterone on adult female paced behavior, the aromatase inhibitor, ATD, was administered to pregnant mothers (and hence her offspring) during prenatal development through parturition using the same the gestational exposure periods as in the previous two experiments. Genital morphology of the offspring was measured at birth. Animals were tested for paced copulatory behavior in adulthood. If the ATD successfully blocks the conversion of testosterone to estrogen in females, alteration of the pacing of sex behavior could be expected in adulthood.

Methods

Pregnant females were implanted with ATD filled silastic capsules beginning at day 10 of gestation. A total length of 120mm of ATD capsule surface was implanted sub-cutaneously into the nape of the neck. Several small capsules were needed to attain the total 120mm length of ATD capsule surface. The set of capsules implanted consisted of six individual capsules each with 20mm of ATD capsule surface. Each capsule was pricked 75 times with a 25 gauge injection needle prior to insertion to further aid in the release of ATD.

RESULTS

Morphological Measures

The genital morphology of female offspring was affected by prenatal exposure to the aromatase inhibitor ATD. Anogenitial distances of newborn females exposed prenatally were significantly larger than controls (Figure 11) (t-test, $p<0.05$) and were similar in anogenital distance to males in control litters. Males exposed prenatally to ATD also showed a significant increase in anogenital distance when compared to control males (not shown). Body weights were not significantly different between ATD exposed and control offspring of either sex.

Adult Sex Behavior

Female pacing behavior was affected by prenatal exposure to ATD. Females exposed to ATD prenatally had a higher intromission rate than controls (Figure 12)(t-test, $p<0.05$). Following receipt of an ejaculation, prenatally ATD exposed females did not leave the male's chamber as often as was seen in controls, hence in a majority of cases their post-ejaculatory refractory period was abolished (Figure 13) (Chi-Square, $p<0.05$).

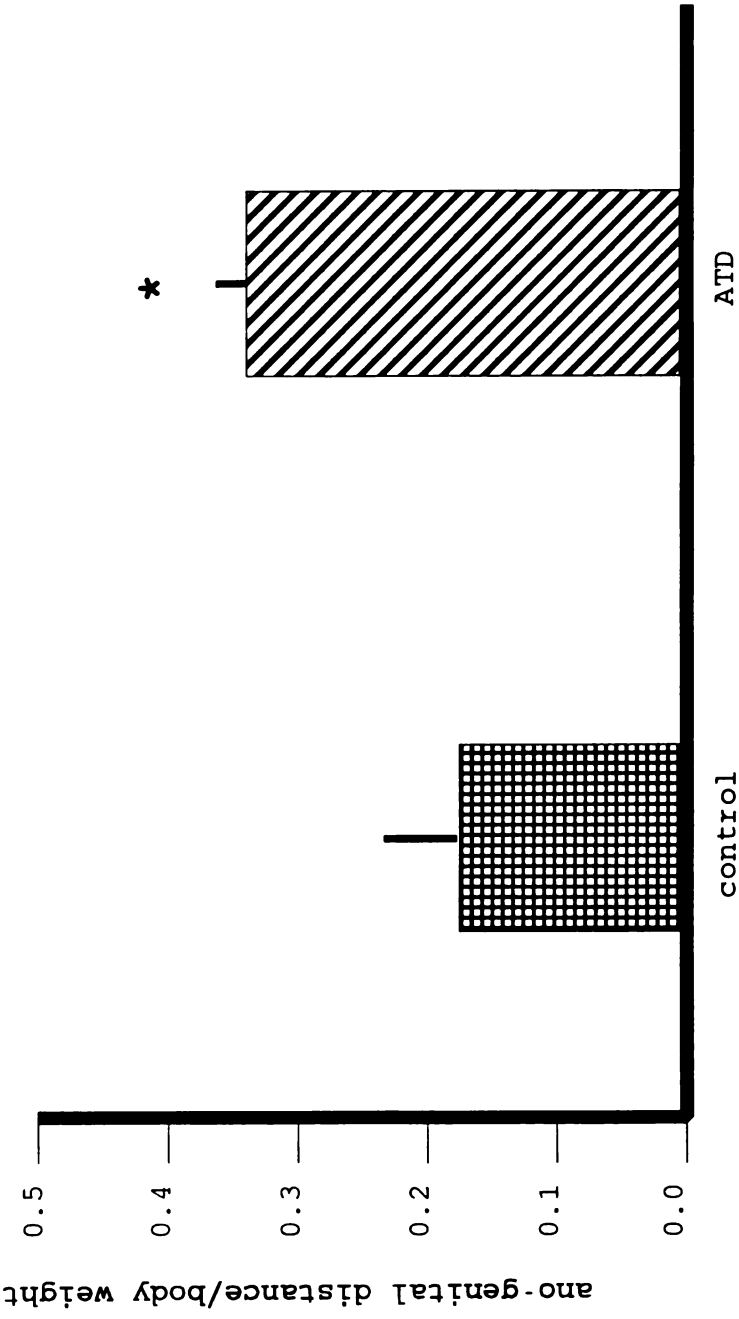

Figure 11. Comparison of control and ATD treated females in their ano-genital distance/body/weight (t-test, $p<0.05$).

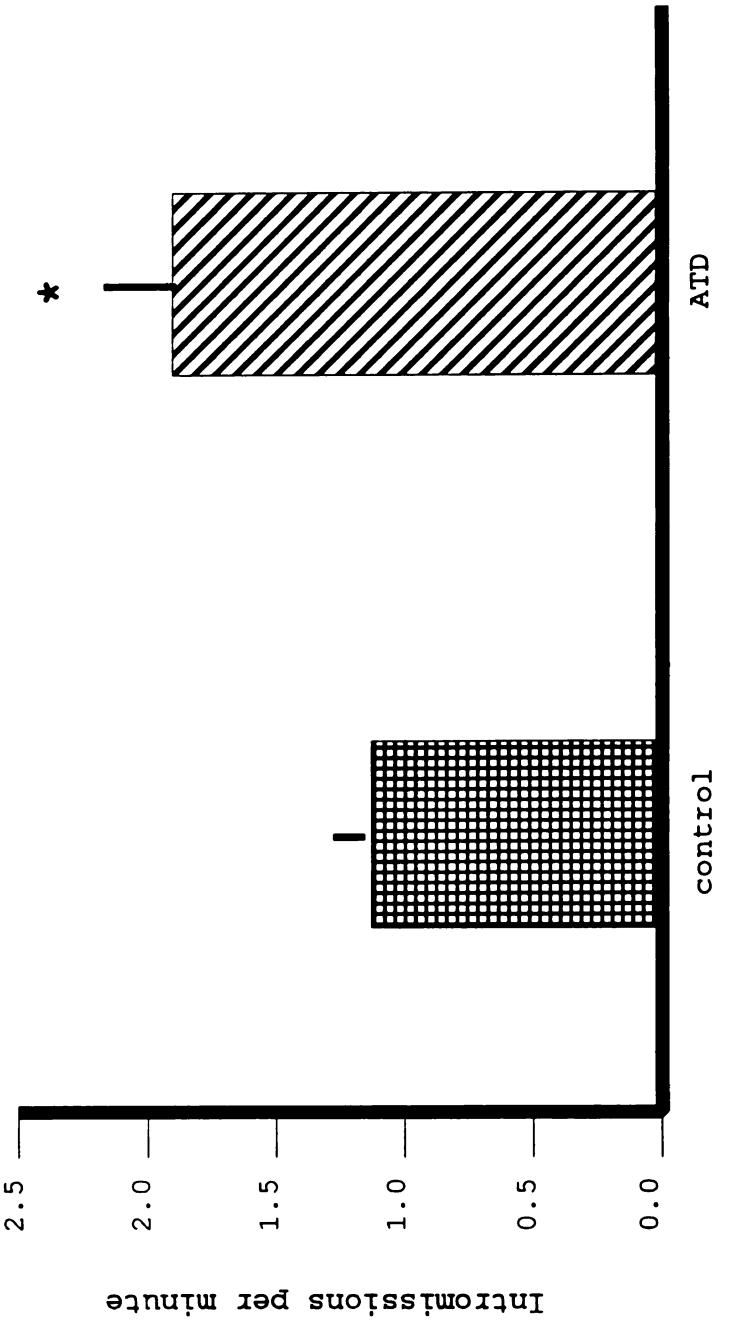

Figure 12. Comparison of intromission rates in control and ATD treated females (t-test, $p<0.05$).

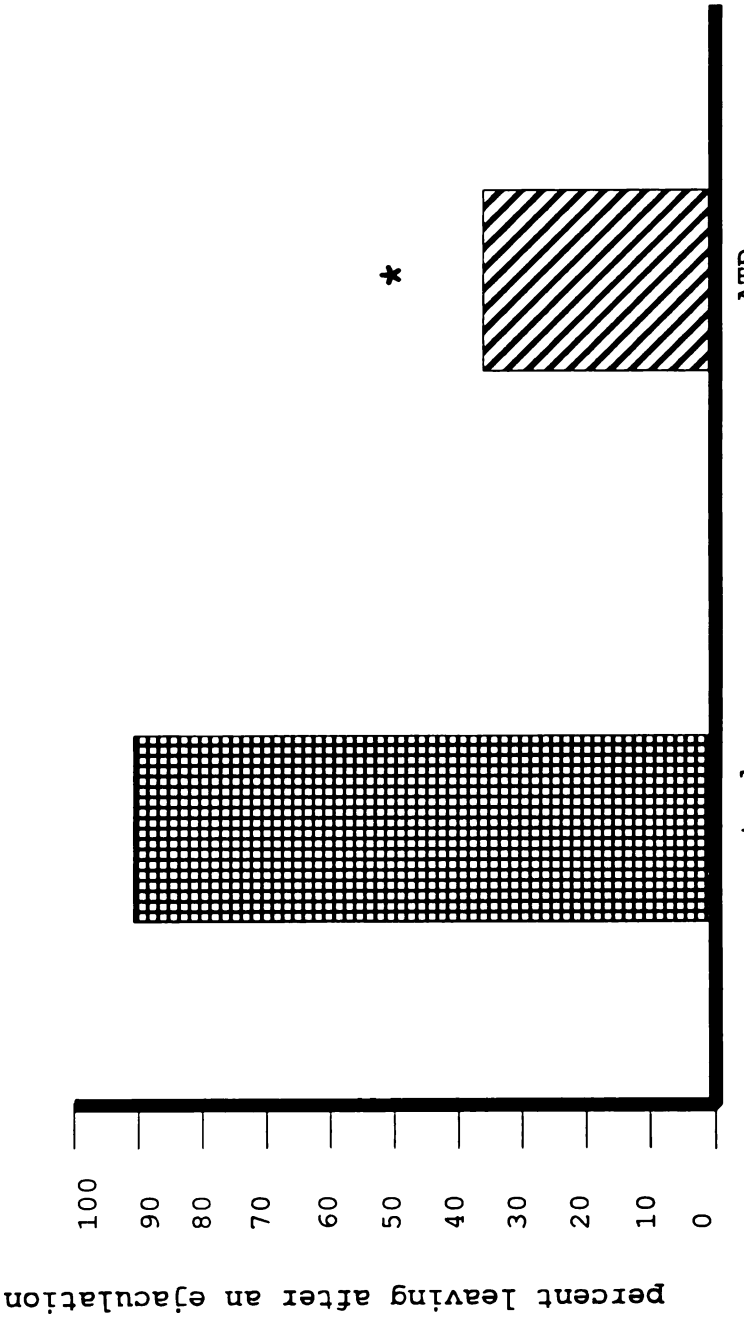

Figure 13. Comparison of refractory periods in control and ATD treated females after receiving one ejaculation (Chi-Square, $p<0.05$).

DISCUSSION

The most consistent findings in these studies were the increase in intromission rates and the loss of the female's post-ejaculatory return latency in the majority of androgen exposed females. While normal females typically leave the male's chamber after ejaculation, most females prenatally exposed to testosterone or androsterone stayed in the chamber with the male.

Additional findings confirmed the previously reported lack of effects of prenatal exposure to androgens on female lordosis quotients (Houtsmuller & Slob, 1990). No significant differences in lordosis quotients were seen between control females and females exposed prenatally to androgens when tested.

Finally, the third experiment revealed that females exposed prenatally to ATD also showed increases in intromission rates and nearly complete abolition of post-ejaculatory return latencies. These results paralleling androgen exposed females were unexpected and do not appear to mesh well with the prior two studies presented on androgen exposure. However, explanations are presented that help resolve these seemingly contradictory data.

Organization and Development of the Timing of Female Sex Behavior

While the concept of female sexual behavior being dependant upon prenatal exposure to gonadal steroid hormones is not new, no prior investigations have demonstrated a relationship between the timing of adult female sexual behavior and prenatal exposure to androgens. A key question concerns the mechanism(s) by which androgen exposure effected this change in behavior. Is this organizational effect an indication of central or peripheral changes in the development of the nervous system? While central effects cannot be ruled out at this time, intriguing evidence supports the idea that the effects are of a peripheral origin.

The pelvic ganglia is a major ganglia in the rat associated with sensory feedback from the genital region of both sexes. The pelvic and pudendal nerves, are involved in the sensory components that are important for sexual behavior. However, the role for each nerve appears distinctly different. In the normal female, the pelvic nerve innervates the area of the vagina near the cervix (Peters et al., 1987) and the cervix itself (Komisaruk et al., 1972), whereas the pudendal nerve carries sensory stimulation primarily from the clitoris, the external areas surrounding the vaginal opening, internal areas near the

entrance to the vagina, and the anal and urethral sphincter muscles in the rat (McKenna & Nadelhaft, 1986).

Thus, deep internal stimulation received by the female during copulation is primarily transmitted via the pelvic nerve. This deep stimulation would be most pronounced at the time of receipt of an ejaculation. Electrical stimulation of the pelvic nerve has been associated with increased intra-vaginal pressure in the female (Pacheco et al., 1989) suggesting ejaculatory behaviors received by the female largely stimulate the pelvic nerve.

Intromissions are associated with stimulation of the vaginal opening and the caudal wall of the vagina (Adler, 1977), although intromissions also result in stimulation of the vagino-cervical regions. Receipt of intromissions, therefore, causes high levels of stimulation of both the pudendal and pelvic nerves in females.

In the present research, androgen exposure was associated with an increase in intromission rate as well as an apparent decrease in sensitivity to ejaculation (as suggested by the low number of post-ejaculatory return latencies). Lodder and Zeilmaker (1976) have suggested that pudendal nerve stimulation is a key factor in the motivation of female sexual behavior, and the present findings of increased intromission rates suggest that motivation to engage in sex behavior has not been abolished by androgen exposure. The increased intromission rate may, however, be

an indication of pelvic nerve changes. Likewise, loss of the post-ejaculatory refractory period in many females suggests that the innervation by the pelvic nerve may have been altered by androgen exposure. The idea of androgen sensitivity for the tissues of the pelvic ganglia is supported by the known sexually dimorphic differences in the size of this ganglia. Males have a nearly three-fold greater number of neurons making up the pelvic ganglia than do females (Greenwood et al., 1985). If pelvic nerve innervation of the tissues of the vagino-cervical area were altered by prenatal treatment with androgens, it is possible that this change modified sensory input.

Prenatal Androgen Exposure and Lordosis Quotient

No known direct effect has been previously reported for prenatal androgen exposure on female lordosis quotient. However, indirect evidence for an effect of androgen was suggested by Gladue in 1979. Gladue found that prenatal administration of the anti-androgen flutamide, resulted in significant increases in lordosis quotients in females. With the idea of an anti-androgen promoting lordosis quotient, it is logical to conclude that high levels of prenatal androgens would be associated with decreased lordosis in females. This predicted decrease in lordosis quotient was not supported by the present findings.

In the present study, androgens did not decrease lordosis. Lordosis quotients were very high in females exposed prenatally to testosterone or androsterone and were not significantly different from the equally high lordosis quotients expressed in control females.

This seeming disagreement between the research presented here and the research of Gladue, however, may simply be an artifact due to the different vehicles used for administration of the exogenous treatments. Gladue used propylene glycol as the vehicle in his studies, whereas the present research used sesame oil. Propylene glycol is a physiologically harsh compound and the overall lower levels of lordosis quotients reported for his control animals may indicate some effect of this vehicle. In comparing the control groups of this research (animals receiving a sesame oil vehicle) with Gladue's control groups (animals receiving propylene glycol), there is a pronounced difference in the lordosis quotients reported. In the current study, control females showed lordosis quotients averaging in the upper 90s whereas Gladue's control animals exhibited lordosis quotients closer to 70. With the probable stress of the vehicle used in the work by Gladue, it is likely that the difference between his data and the data presented here results from use of different vehicles.

ATD as an Androgen

The present findings for the effect of ATD do not conform well to the anticipated effects of ATD. ATD, as an aromatase inhibitor, was expected to diminish the conversion of testosterone into estrogen. The use of ATD in this work was an attempt to lower the possibility of an estrogenic effect due to naturally circulating aromatizable androgens. Limiting the conversion of estrogen from testosterone would support the idea for androgenic action of gonadal hormones on the timing of female sexual behavior, and because testosterone levels are normally quite low in the developing female, it was anticipated that no effect on the timing of sexual behavior would be seen. Instead, the results presented show the same effect for prenatal ATD exposure as was discovered for exogenous testosterone or androsterone: an increase in the intromission rate and very often the abolition of the female's post-ejaculatory return latency. Two explanations for these results are plausible.

First, prenatal exposure to ATD in these females may have resulted in a rise in the naturally occurring androgens in circulation, because little if any testosterone would have aromatized into estradiol. It is possible that some threshold level of androgen concentration necessary for the alteration of timing of sexual behavior in the female was reached. Under most conditions this threshold would not be

attained due to the interplay of the aromatase enzyme helping to keep levels of endogenous testosterone low.

A second possibility is that the prenatal ATD exposure these females received exerted its own androgenic effect on development. At high doses, ATD has been shown to have androgenic-like activity (Bakker et al., 1993; Brand et al., 1993) and the exposure to ATD that females in this study received was great due to the total length of the ATD filled capsule, and also due to the method of puncturing the capsules to increase flow.

Regardless of the actual mechanism involved, the current findings cannot entirely rule out a role for prenatal exposure to estrogen in the timing of sexual behavior in the female, but these data support the idea that androgens prenatally influence timing of adult sex behavior in female rats.

The role gonadal hormones play in the organization and activation of adult behavior is relevant to studies of sexual orientation and/or partner preference, but has received relatively little attention until recently (Adkins-Regan, 1988). When speaking of animal studies, it is more accurate to consider partner preference as opposed to sexual orientation, because of the difficulties inherent in studies of motivation in animals. Partner preference is defined as the time an animal spends or interacts sexually with another animal when given the choice to interact between two or more

stimulus animals. Brand et al., (1991) have reported that estrogen derived from the aromatization of testosterone organizes both adult sexual preference and adult sex behavior. Male rats treated neonatally with ATD show diminished preference for an estrous female and showed increased rates of lordosis when paired with a sexually experienced stimulus male (Brand et al., 1991). This neonatal treatment of male rats with ATD has previously been shown to interfere with the differentiation of the central nervous system but not morphological somatic development (Vreeburg & van der Vaart, 1977), and hence the partner preferences are not a result of changes in genital morphology. More recently, it was shown that these males neonatally treated with ATD also show nocturnal rhythmicity in partner preference (Bakker et al., 1993). These males exhibit more strongly the "feminine" behaviors of lordosis and association with stimulus males in the early phase of the dark cycle but display the more "masculine" behaviors of intromissions and ejaculation along with greater association with an estrous female in the later phase of the dark cycle (Bakker et al., 1993).

These findings regarding male partner preference being affected by altering neonatal estrogen levels through ATD may help, in part, to explain the findings of the present study. Normal female rats show some "masculine" sexual behaviors (i.e., mounting) in adulthood, but the variation

of mounting expressed between individual females is large (Slob & Van der Schoot, 1982; Van de Poll et al., 1982). Endogenous variability in prenatal circulating aromatizable androgens may be responsible for the range of mounting behaviors expressed in normal females (Clemens et al., 1978), Exogenous treatment of ovariectomized females with testosterone increases their display of mounting behavior (Slob et al., 1987) and very recently it was found that prenatal females with caudal males in the same uterine horn display higher levels of testosterone at gestational day 19 than do females lacking a caudal male (Houtsmuller et al., 1995).

The abolition of the female's post-ejaculatory return latency in the majority of ATD exposed females in the present study could be an expression of one aspect of altered partner preference displayed by the female. If so, it would be fruitful in future work to examine this alteration of central nervous system development in females by combining both measures of the timing of pacing of sexual behavior as well as in tests designed to address issues of partner preference. Such a combined focus could elucidate greatly the organizing effects of prenatal exposure to the gonadal steroids.

SUMMARY AND CONCLUSIONS

Events leading to the differences of adult masculine and feminine sex behavior occur prenatally and neonatally through the actions of the gonadal steroid hormones, androgens and estrogens. Behavioral development as well as accessory sex structure development in females normally occurs along a continua of feminization, the promotion of female specific sex accessory structures, and demasculinization, the regression of male specific sex accessory structures present in the bipotential zygote. Normal rats display feminine sex behavior in adulthood due to low circulating levels of estrogen produced by the ovaries prenatally, whereas females given testosterone in late prenatal life were masculinized and showed abnormally rapid growth. This suggests that high androgen exposure is atypical for normal female development, but there are naturally occurring situations where females do develop in the presence of androgens. In the uterine horn of the rat, multiple pups of both sex develop in unison within a litter. While each pup has its own placental attachment within this horn, there is exposure to the hormones produced by neighboring pups. Females who develop between two males *in*

utero are exposed to testosterone levels that can cause changes in her adult behavior. Such females show more aggressive behavior and longer ovarian cycles but do not differ from other females in their fertility or litter sizes. These findings suggest that while androgens may not be required for prenatal development of the female, they are present for many females, and the role of androgens in the organization of the female body cannot be simply eliminated or excluded. The present research re-affirms the idea that androgen exposure is a critical parameter in the organization and development of the nervous system of the female rat, and presents evidence for a profound impact of androgens on the timing of female adult sex behavior.

Females exposed to androgens prenatally exhibited an increased lordosis rate and in many females the post-ejaculatory return latency was abolished. It is postulated that the effect androgens exert is through alteration of the organization of the peripheral nervous system, and the likely target site for this effect is the pelvic ganglia.

This study demonstrates the essential role of the prenatal environment in the development of the timing of adult sex behavior in rats and reiterates the need for consideration of this prenatal environment when evaluating the potential for the timing of rat adult sex behavior. Many dimorphic differences between the sexes arise during this prenatal period, and the timing dimorphism during adult

sex behavior is one way in which the still cloudy view of sex differentiation is further clarified.

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