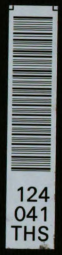

This is to certify that the

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Patricia Hilley Ruppert

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THE ROLE OF AROMATIZATION IN THE DEVELOPMENT OF SEXUAL BEHAVIOR IN THE HAMSTER (MESOCRICETUS AURATUS)

By

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ABSTRACT

THE ROLE OF AROMATIZATION IN THE DEVELOPMENT OF SEXUAL BEHAVIOR IN THE HAMSTER (MESOCRICETUS AURATUS)

By

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A major factor determining the potential for masculine or feminine sexual behavior is the action of gonadal hormones during early life. Males exposed to testicular hormones during sexual differentiation are masculinized and defeminized in their behavior as adults. Female hamsters, or males castrated on the day of birth (Day 1), if exposed to androgens which can be converted to estrogens or estrogens themselves during early development, also show an increase in masculine behavior and a decrease in feminine behavior.

The present study investigated whether aromatization of testosterone to estradiol is necessary for behavioral masculinization and defeminization. An aromatization inhibitor, ATD (1,4,6-Androstatrien-3,17-dione) was administered to intact male and female hamsters, and to Day 1 castrate males along with either testosterone or estradiol. On days 2 through 4 after birth, all animals received either 1.0 or 0.5 mg ATD or propylene glycol along with either 50 or 100 µg testosterone, 2 µg estradiol or sesame oil. As adults, all were tested for both masculine and feminine sexual behavior in counter-balanced order.

In Experiment 1, both testosterone and estradiol masculinized the behavior of females and Day 1 castrate males. Neither animals receiving ATD alone nor the control animals were masculinized. The

high dose of ATD (1.0 mg) completely blocked masculinization produced by the low dose of testosterone (50 μg) for females and Day 1 castrate males. The high dose of ATD (1.0 mg) partially blocked masculinization produced by the high dose of testosterone (100 μg) in females but not Day 1 castrate males; ATD, as predicted, did not block behavioral masculinization produced by estradiol. For intact male hamsters given postnatal hormones, males receiving a combination of 1.0 mg ATD + 100 μg T intromitted and ejaculated more than males just receiving 1.0 mg ATD + oil; ATD did not block the combined effect of endogenous and exogenous testosterone in intact males.

For female behavior in Experiment 1, control females receiving ATD + oil showed longer mean lordosis durations than females receiving a high dose of testosterone or estradiol, with or without ATD. Feminine sexual behavior subsequent to treatment with 1.0 mg ATD + 50 μg T was intermediate between the lordosis durations of the controls and females defeminized by testosterone or estradiol. Although both intact males and Day 1 castrate males were more defeminized than female hamsters, regardless of hormone treatment, there were no significant differences within male treatment groups.

Since, for each animal the order of testing for masculine and feminine behavior was counter-balanced as a control procedure, the data were analyzed for order effects; significant differences were found for order of testing. For some treatment groups, female hamsters, intact males or Day 1 castrate males who received masculine behavior tests prior to feminine behavior tests showed higher masculine behavior scores than animals tested in the reverse sequence.

For feminine behavior in females, order effects were also seen. While higher masculine scores for postnatally intact males receiving masculine behavior tests first may reflect a residual effect of testicular hormone secretion by the adult gonad, the same finding in females and Day 1 castrate males, however, poses a challenge to the present concept of sexual differentiation.

In Experiment 2, females receiving either testosterone or estradiol postnatally, either with or without ATD, were tested for feminine behavior as adults to determine their sensitivity to ovarian hormones. With acute doses of estradiol ranging from 1.5 to 12 μg estradiol benzoate, females showed little or no lordosis in the absence of progesterone. Progesterone in doses ranging from 50 to 400 μg facilitated receptivity for all groups when combined with priming doses of estrogen; no differences were seen between postnatal treatment groups. In Experiment 2, as in previous behavioral studies in the hamster, evidence does not indicate that early hormone treatments influence adult hormone sensitivity.

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INTRODUCTION

For the hamster, estrogens as well as aromatizable androgens induce the potential for masculinization and defeminization of behavior when present during the first few days after birth. These behavioral studies led to the hypothesis that testosterone is a "prohormone", achieving its biological potency via its metabolites of estradiol and dihydrotestosterone. The purpose of the present study was to investigate whether testosterone must be metabolized to estradiol for hamsters to be behaviorally masculinized and defeminized. This hypothesis was tested by giving ATD (1,4,6-Androstatrien-3,17-dione), which inhibits the aromatization of testosterone to estradiol, to male and female hamsters, in combination with either testosterone or estradiol. The specific hypotheses of Experiment 1 were:

1) Behavioral masculinization of male and female hamsters is accomplished via conversion of testosterone to estradiol. Therefore, ATD, which blocks aromatization, will block the behavioral masculinization of male and female hamsters by testosterone but will not block estradiol induced masculinization.
2) Defeminization of behavior is also mediated by estradiol. Male and female hamsters given a combination of ATD and testosterone will show normal feminine behavior while those

receiving ATD and estradiol will show a reduced duration of lordosis.

Defeminization of behavior may involve changes in sensitivity either to estrogen, to progesterone or to both hormones in adulthood. In Experiment 1 of this study, and in other developmental studies, female hamsters were tested for lordosis with hormone treatments which would maximize behavioral responding. The purpose of Experiment 2 of this study was to obtain dose response data for lordosis in both normal and defeminized hamsters using adult hormone treatments of estrogen alone and estrogen plus progesterone. These dose response data for lordosis in the female hamster should provide information on whether behavioral defeminization involves a change in estrogen sensitivity, progesterone sensitivity, or whether both are changed.

The golden hamster is a good model for studies of reproductive biology and behavior. Because of its short gestation period, only 16 days, the hamster is born at an immature stage. Therefore, developmental processes which occur prenatally in most species occur postnatally in the hamster. As a result of this immaturity, hormone treatments which would reach the fetus indirectly in other species, via the mother, can be given directly to hamster pups. The hamster, then, offers a comparative model for determining physiological processes regulating behavior. In this study, the goal was to elucidate one aspect of the mechanism of early hormone action, using the hamster as a model.

In the introduction which follows, the rationale for these experiments is described. The first section contains a description of the behavior patterns which characterize masculine and feminine

sexual behavior in the adult hamster. Then the organizational model of sexual differentiation is presented for behavior, morphology and patterns of gonadotropin release. Further discussion summarizes experiments on the development of masculine and feminine sexual behavior in the hamster. Then I review the physiological evidence for aromatization, and show how ATD can be used to test the hypotheses of the present experiments.

Adult Masculine Sexual Behavior

Masculine sexual behavior in the hamster consists of a series of mounts, intromissions and ejaculations; hamsters are classified as a multiple intromission, multiple ejaculation species. Copulatory behavior in the hamster has been well described by Reed and Reed (1946), Beach and Rabedeau (1959) and by Bunnell, Boland and Dewsbury (1977). The account presented here follows from their behavioral studies. Also, Figure 1 is a schematic diagram of masculine sexual behavior in the hamster, showing the behavioral components of copulation, and the temporal pattern of behavior.

When paired with a receptive female, the first response of the male is to sniff and lick the head, body and particularly the genital region of the female. Then, during a rear mount, the male clasps the female with his forelegs, elevates his pelvis and thrusts rapidly. Occasionally, males especially inexperienced ones, mount the female from the head and side; in this case, the male either reorients himself spontaneously on subsequent mounts or the female shifts her position so he is oriented to her rear. After a few mounts, the male intromits, i.e., the penis penetrates the vagina while the male is

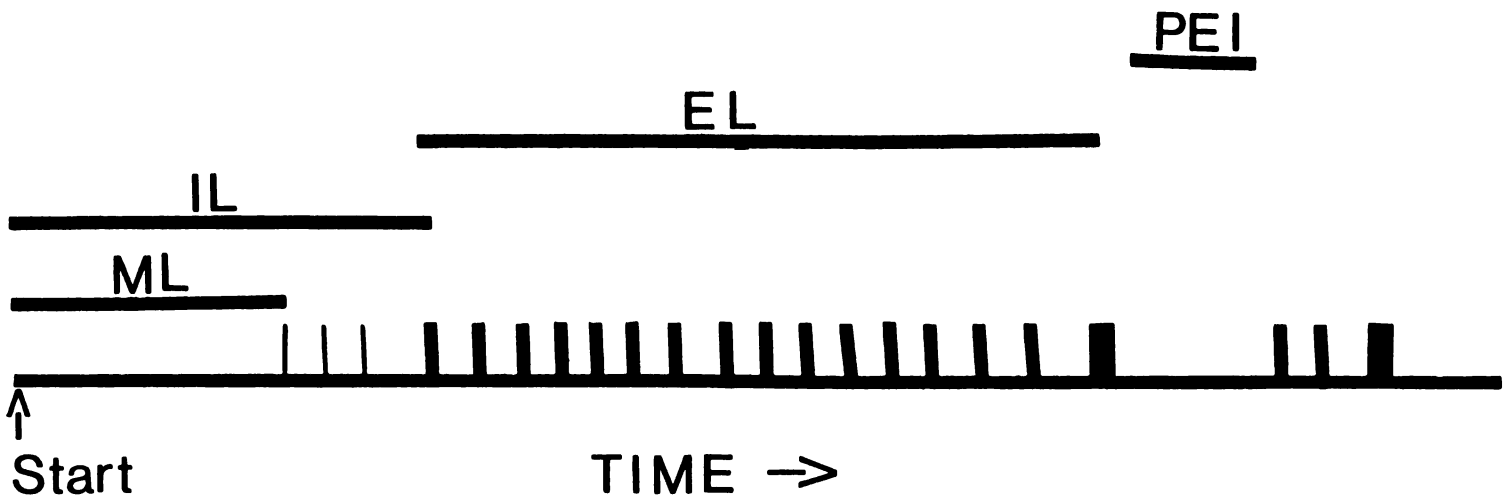

Figure 1. Schematic diagram of masculine sexual behavior in the hamster showing mounts (thin lines), intromissions (medium lines) and ejaculations (thick lines). The temporal relationships are depicted above the time line; ML (mount latency), IL (intromission latency), EL (ejaculation latency) and PEI (post-ejaculatory interval) are shown.

mounting. During intromission, the male thrusts rapidly and then brings his pelvis firmly against the female's perineum.

After about 15 intromissions, spaced 10 seconds apart, the male ejaculates. Behavioral criteria for ejaculation (intromission plus sperm emission) are subtle in the hamster. The best indication of ejaculation is an increase in the rate of pelvic thrusting prior to intromissions. An additional behavioral criterion is a spasmodic flexion and extension of the rear leg during ejaculation. While intromissions and ejaculations differ in average duration (2.4 vs. 3.4 seconds), this is difficult to distinguish behaviorally.

Figure 1 also indicates the temporal patterning of the male's behavior. The mount latency is the interval from the introduction of the female to the first mount, and the intromission latency is the interval from the introduction of the female to the first intromission. These time periods are quite variable, both between animals and on different tests with the same animal. The preliminary exploratory activity of both partners, and the initial orientation of the partners to one another contribute to this variability. The median mount latency is about one minute, with an intromission latency of 1.5 minutes.

Once the male begins to copulate, his behavior is very channelized. Prior to the first ejaculation (the ejaculation latency is the interval from the first intromission to the first ejaculation), 80% of the male's behavior is spent in pursuit-mount of the female or in genital grooming; males lick their penis and groom following intromission. During the post-ejaculatory interval (the time from ejaculation to the next intromission), which is about 30 seconds, males

groom in longer bouts (at least 6 seconds), and then explore the surrounding area or sniff and lick the female. These post-ejaculatory behaviors are additional indicators that ejaculation has occurred.

After the first ejaculation, the temporal pattern of behavior changes. The ejaculatory latency decreases from about 3 minutes for the first ejaculation to 30 seconds for subsequent ejaculations. The number of intromissions decreases dramatically from 15 to 3, and the time between intromissions decreases slightly (the male shows shorter bouts of grooming). As the male approaches satiation, after 9-10 ejaculations, the time between intromissions increases slightly, while the post-ejaculatory interval increases progressively during the test period. By the last series, only 52% of the male's activity is spent in pursuit-mount or genital grooming; locomotory-exploratory behavior, licking the female, biting the female, lying down, grooming and scratching are more prevalent.

Female hamsters have a very limited capacity to display components of masculine sexual behavior unless treated postnatally with gonadal hormones. In all developmental studies where control females received oil injections, none of the females mounted as adults with any of the adult hormone treatments. Tiefer (1970) tested normal adult females for masculine behavior under three conditions; natural estrus, estrogen plus progesterone and testosterone treatment. Only 1/10 females mounted and showed the intromittive pattern. The conclusion drawn from these studies was that female hamsters are not bisexual; they do not behave as males when given appropriate hormone treatments as adults.

Noble has challenged this conclusion. He tested females for masculine behavior using several combinations of adult hormone treatments (Noble, 1974). While neither estradiol benzoate, dihydrotestosterone propionate or testosterone propionate alone induced mounting in females (0, 29 and 33% mounted, respectively), with combined treatment with estradiol benzoate and dihydrotestosterone propionate, all females mounted and most intromitted. Hormone doses were extremely high: 6 μg estradiol benzoate plus 1-2 mg dihydrotestosterone propionate were given daily, and the median latency to onset of mounting was 21-31 days after the beginning of hormone treatment. Nonetheless, adult females can perform the major components of masculine copulatory behavior except for the ejaculatory pattern. They require high doses of combined hormone treatment for an extended period of time, however, to elicit masculine behavior.

With silastic implants of hormones, producing a more physiological pattern of hormone release, Noble (1977) found that either testosterone alone or a combination of either testosterone, dihydrotestosterone or androstenedione with estradiol was effective in inducing mounting and intromitting in females. Again, latencies to the first mount were long, 31-35 days. Noble hypothesized that the neural structures underlying masculine copulatory behavior are organized in both males and females; females, though, are more refractory to hormones. They may be less able to convert testosterone to its metabolites. Extended, high doses of hormones in adult females might be ".....producing changes similar to those which typically occur during exposure to gonadal steroids early in development" (Noble, 1977, p. 520).

Adult Feminine Sexual Behavior

The feminine sexual behavior of hamsters is unique to rodents. When a receptive female is placed with a male, he usually sniffs or licks her a few times. She then takes a few steps away from his and assumes the lordosis posture. "She extends her forelegs but flexes them slightly at the elbow, extends the hind legs and elevates the pelvis, elongates the body and straightens the back, spreads the hind feet wide apart and raises her tail. The eyes are glazed, fixed and may be half closed" (Reed & Reed, 1946, p. 8).

Beach has shown that female hamsters do behave proceptively, i.e., females in estrus solicit male attention. Receptive females spend more time near a caged male than diestrous females, and they remain in tonic immobility or full lordosis in the presence of sensory cues from the male (Beach, Stern, Carmichael & Ranson, 1976). In a mating test, contact with the male occurs almost immediately and the female does not have the opportunity to solicit. Since tactile cues alone are sufficient for females to show lordosis (i.e., manual stimulation, Murphy, 1974), and females will show lordosis to the auditory, visual and olfactory cues of a caged male, the sensory cues available in a mating test are redundant and the female readily shows lordosis.

Females remain in a trancelike state for long periods of time while the male copulates. Even during intervals when the male is not actively copulating (i.e., during genital grooming), females remain immobile. During the first 5 ejaculation series of the male, lordosis and walking lordosis are the only behaviors of the female (Bunnell et al., 1977). Measures of feminine receptivity in the hamster are

total lordosis duration (total time during a test spent in lordosis), lordosis frequency (the number of times the female shows lordosis), and the longest duration of lordosis (the longest single lordosis episode during a test).

After prolonged genital stimulation, or following the maximum period of behavioral estrus, females become less receptive to the male (Carter, Landauer, Tierney & Jones, 1976; Carter & Schein, 1971). Intact females are receptive for 14 hours if not mated; proceptive behaviors are characteristic of the middle 8 hours of estrus (Beach et al., 1976). As the female becomes unreceptive, she shows lordosis only while the male is actively copulating; during pauses in copulation, she explores the testing arena. With additional genital stimulation or passage of time, the female actively resists the male's attempts to mount by turning on her side or biting. Females who are initially unreceptive also attack if the male persists in attempting to mount.

Since male hamsters are normally bisexual, feminine sexual behavior is relatively easy to elicit, even in the intact male. Kow, Malsbury and Pfaff (1976) investigated lordosis in intact males using manual stimulation. The lordosis potential of males is greater when receiving manual stimulation than when mounted by another male hamster; the extent of tactile contact is greater and the attractiveness of the experimental male is not a factor. (Since the male showing lordosis lacks a vagina and cannot receive intromissions, he is not a prized partner; the stimulus male may cease mounting or become aggressive after many unsuccessful attempts to copulate.) The total lordosis duration of intact males in these tests with manual stimulation was

half that of receptive females' (57.2 vs. 109.2 seconds). When males showed lordosis, the testes were withdrawn toward the body cavity, furthering the similarity to the female. While castration did not decrease the lordosis shown by males, treatment with estrogen and progesterone increased lordosis duration in males to the equivalent of the estrus females .

When adult males are castrated and their feminine sexual behavior compared to females', males behave qualitatively similar but differ in the frequency and duration of lordosis. While females hold a single lordosis during the male's mounts and dismounts, males tend to remain in lordosis only while the stimulus male is actually mounting. This results in a more frequent but shorter duration of lordosis (an increase in lordosis frequency but decrease in lordosis duration).

Males castrated without further hormone treatment do not show lordosis in mating tests. With short term exposure to estrogen alone (1 or 2 injections) the data conflict on whether males show lordosis (Carter, Michael & Morris, 1973; Tiefer & Johnson, 1971); lordosis, if shown to short term estrogen alone, is very brief. Long term exposure to estrogen (6 days or more) is much more effective in inducing behavioral receptivity (Carter et al., 1973), and progesterone facilitates receptivity with either short term estrogen (Carter et al., 1973; Tiefer & Johnson, 1971) or long term estrogen treatment (Carter et al., 1973).

Sexual Differentiation

In a now classic study in behavioral endocrinology, Phoenix, Goy, Gerall and Young (1959) proposed that hormonal conditions during

early life organize sexual behavior in a masculine or feminine pattern, just as hormones organize the reproductive system and gonadotropin release into a masculine or feminine pattern. This inference was based on the behavior of guinea pigs treated with testosterone propionate during development. According to this hypothesis, during a restricted, critical period of early life, gonadal hormones organize neural tissues to facilitate the sexual responses of the genetic male and to inhibit or suppress the sexual response patterns characteristic of the genetic female. This is the condition of the normal male and of females receiving hormones during the critical period. In the absence of gonadal hormones, the condition of the genetic female or the post-natally castrated male, the potential for feminine behavior develops.

This hypothesis initially recognized that behavioral masculinization and defeminization were independent and separable processes. Beach has defined masculinization and defeminization broadly to include a range of sexual differences: masculinization refers to the induction of anatomical, physiological or behavioral characteristics typical of males but lacking or poorly developed in females, while defeminization is an inhibition of characteristics well developed in females but not in males (Beach, 1975). For each species or strain investigated, either the male or the female in each case is likely to be bisexual; for hamsters, for example, female hamsters rarely mount as adults but male hamsters easily show feminine sexual behavior. Rather than a "critical period" when hormones can modify the potential for bisexuality in each species, early life can more aptly be considered a "period of maximum sensitivity", during which gonadal hormones exert their organizational effects (Goy & Goldfoot, 1975).

Besides behavioral sexual differentiation, morphological and neuroendocrine sexual differentiation are also influenced by the action of gonadal hormones during early life. The successive steps in morphological sexual differentiation are: genetic sex, gonadal sex and then morphological sex. Jost (1953, 1972) first obtained evidence supporting this model of development by removing the gonads from male and female rabbit fetuses before the stage of morphological differentiation. Regardless of genetic sex, all gonadectomized fetuses developed as females. Jost's interpretation, that testicular secretions induce the male phenotype, and that the female phenotype results from the absence of testicular hormones, has been supported by subsequent gonadal transplant studies and by studies using exogenous gonadal hormones.

At the stage during which the undifferentiated gonad is developing into an ovary or testis, both sexes have a urogenital system composed of two parts: 1) a dual duct system (mullerian and wolffian) which is the anlage of the accessory sex organs and the upper vagina, and 2) the urogenital turbercle which is the anlage of the external genitalia. The fetal testis produces two substances necessary for male development (Wilson, 1978). The first is an unknown peptide (mullerian inhibiting substance) which causes the mullerian ducts to regress. The second is testosterone which induces maturation and development of the wolffian duct system (forming the vas deferens, seminal vesicles, epididymis, and accessory glands), the external genitalia and the testis itself.

Jost (1953) found that differentiation of the mullerian duct system developed in the absence of the fetal ovary, and concluded

that ovarian secretions were not involved in female development. In the absence of fetal testicular secretions, the wolffian ducts regress and the mullerian ducts develop to form the uterus, oviducts and upper vagina; the genital tubercle forms the clitoris and external vagina. However, the embryonic ovary can synthesize estrogen (Milewich, George & Wilson, 1977) and local estrogen formation may aid on the maturation of the ovary.

In the hamster, morphological sexual differentiation begins on Day 10 of gestation and continues throughout the early postnatal period. First indications of testicular differentiation begin at Day 11 3/4, while the ovary begins to develop a day later. The wolffian ducts appear at Day 10 and continue to develop in males and females until Day 13 3/4 when degeneration begins in the female. The mullerian duct is formed in both sexes by Day 11 3/4 and begins to degenerate 24 hours later in the male. Although the genital tubercle first appears at Day 9 3/4, development of the external genitalia continues postnatally (Ortiz, 1945).

Just as behavioral and morphological sexual differentiation are determined early in life by the action of gonadal hormones, hypothalamic control of gonadotropin release in rodents is programmed in a masculine (acyclic) or feminine (cyclic) pattern by the hormonal events in early life. Gorski (1971, 1973) has reviewed the evidence from his laboratory and others leading to this conclusion. In the absence of active stimulation by gonadal hormones, as in the normal female, the ovariectomized female and the castrate male rat, it has been proposed that the preoptic area-anterior hypothalamus differentiates into a region controlling cyclic release of gonadotropin.

If gonadal hormones are present during early life, as in the normal male or androgenized female, this cyclic center is suppressed and the surge of gonadotropin which induces ovulation does not occur in the adult. In both males and females the control system of the mediobasal hypothalamus is operative, maintaining testicular function and ovarian follicular development. But only in males and females who have not been exposed to gonadal hormones postnatally does the cyclic control system of the preoptic area-anterior hypothalamus develop.

Development of Sexual Behavior in Hamsters

Behavioral studies on the development of sexual behavior in hamsters have sought to define the role of hormones in early life. These studies have shown that testosterone, other aromatizable androgens and estrogens masculinize and defeminize the behavior of male and female hamsters, while non-aromatizable androgens do not have this behavioral potency. The appropriate hormones must be present during the first few days of postnatal life to achieve these behavioral effects. Behavioral studies in the hamster have provided the first evidence that aromatization of testosterone to estradiol is involved in sexual differentiation. This discovery led to the aromatization hypothesis, stating that estradiol is the active hormone in behavioral sex differentiation. The experiments leading up to this hypothesis and to the present study are examined below.

Testicular hormones are necessary for inducing masculinization during early life. Castration of male hamsters on Day 1 of postnatal life eliminates postnatal exposure to gonadal secretions. Thus, males are only exposed to their own testicular secretions during prenatal

development when they are presumably not behaviorally sensitive to the action of hormones (Nucci & Beach, 1971). Castration of male hamsters on Day 1 of postnatal life eliminates the potential for masculine behavior, even if replacement hormones are given in adulthood (Carter, Clemens & Hoekema, 1972; Eaton, 1970; Johnson, 1975; Noble, 1973; Swanson, 1970, 1971). Noble (1973) found that some Day 1 castrates mounted and intromitted while in other studies, males never mounted.

The capacity of Day 1 castrates to behave as males in adulthood can be restored by testosterone replacement during the first few days after birth. This treatment increases the mounting and intromitting of Day 1 castrates (Coniglio & Clemens, 1976; Coniglio, Paup & Clemens, 1973a; Eaton, 1970; Swanson, 1971; Tiefer & Johnson, 1975). None of these studies with either a single injection or multiple injections of hormone over a short time period were able to replicate the full sexual repertoire of the normal male. Tiefer and Johnson (1975), however, gave either testosterone or androstenedione on Days 1-20; their Day 1 castrates resembled adult castrates in mounting and intromitting, but these males ejaculated infrequently.

Although it is difficult to induce mounting behavior in normal female hamsters, postnatal exposure to gonadal hormones induces the potential for masculine behavior in the female. The development of the male has been "mimicked" by injecting female hamsters after birth with either testosterone or testosterone propionate, which has a longer duration of action. For both forms of testosterone, the females so treated have been masculinized, i.e., have mounted as adults (Carter et al., 1972; Coniglio & Clemens, 1976; DeBold &

Whalen, 1975; Johnson, 1975; Paup, Coniglio & Clemens, 1972; Swanson, 1971; Tiefer & Johnson, 1975; Whitsett & Vandenbergh, 1975).

The time period for hormonal exposure and the dose of hormone have been varied to determine the most sensitive period for behavioral masculinization. In comparing different days of castration, Carter et al. (1972) showed that males castrated on Day 6 or 25 mounted and intromitted more than males castrated on Day 1. Noble (1973) found that males castrated on Day 1 only mounted, while Day 5 castrates also intromitted and only Day 10 castrates ejaculated as adults. For females also, the sensitive period for behavioral masculinization by exogenous gonadal hormones is restricted to a fairly short time following birth: 0-72 hours (Swanson, 1971), 1-4 days (Coniglio & Clemens, 1976) or 1-7 days (DeBold & Whalen, 1975). Behavioral masculinization, then, is accomplished within the first week after birth in the hamster.

To determine threshold quantities of hormones needed for behavioral masculinization, Coniglio and Clemens (1976) varied the timing and dose of testosterone given to Day 1 castrate males. 100 µg testosterone either on Days 1-2 or 3-4 increased the mounting and intromitting of castrate males. With a lower dose of testosterone (50 µg), hormone had to be present continuously from Day 1-10 to increase adult mounting and intromitting. DeBold and Whalen (1975) found that one µg testosterone propionate was as effective as 250 µg testosterone propionate in inducing mounting. In another dose response study, Whitsett and Vandenbergh (1975) found that 90% of females mounted when treated with either 3, 30, 300 or 600 µg testosterone propionate; the number of mounts increased with increasing dose of hormone.

Collectively, these studies have shown that the period of maximum sensitivity can be influenced by the dose of testosterone and the form of exposure (alcohol form vs. testosterone propionate). Small doses of testosterone propionate can masculinize behavior while free testosterone must be present in higher doses or for a longer time period to achieve the same effect.

The development of feminine sexual behavior is believed to be an anhormonal process. Since behavioral sexual differentiation presumably does not occur prenatally in the hamster (Nucci & Beach, 1971), the ovaries can be removed on Day 1 after birth in the female hamster to determine the possible influence of postnatal ovarian secretions on the development of feminine behavior. This experiment has been done and behavioral effects are slight (Gerall & Thiel, 1975; Swanson, 1970). Females ovariectomized on Day 43 maintained the lordosis posture longer than females ovariectomized on Day 1, which indicates enhanced receptivity in post-pubertally ovariectomized females. But, the frequency of lordosis was also greater in females ovariectomized on Day 43, and increased frequency of lordosis indicates reduced receptivity. One problem is that the maximally sensitive period for feminization may be very early (hours) after birth, so any delay in ovariectomy might bypass the critical period.

As for masculinization of females, the first approach in studying defeminization has been testosterone administration during early life to "mimic" the hormonal condition of the normal male hamster. The general conclusion from these studies is that testosterone defeminizes females, i.e., females given testosterone during early life show more frequent but shorter lordosis postures (Carter et al., 1972; Coniglio

& Clemens, 1976; Coniglio, Paup & Clemens, 1973b; DeBold & Whalen, 1975; Gerall, McMurray & Farrell, 1975; Gottlieb, Gerall & Thiel, 1974; Johnson, 1975; Swanson, 1971; Tiefer & Johnson, 1971; Whitsett & Vandenbergh, 1975). Coniglio et al. (1973b) obtained defeminization with testosterone propionate but not free testosterone (25 or 100 µg on Days 2-4). Subsequent studies have shown that when the duration of exposure is lengthened by daily injection, either with 50 µg testosterone on Days 1-10 (Coniglio & Clemens, 1976) or via silastic implants on Days 1-10 (Gerall et al., 1975), free testosterone also defeminizes.

Since testosterone suppresses lordosis in females, castration of males soon after birth should result in a feminine pattern of lordosis. Males castrated on Day 1 show longer lordosis durations than males castrated on Day 3 or 5 (Gerall & Thiel, 1975), Day 6 or 25 (Carter et al., 1972) or on Day 10 (Noble, 1973). When males, either neonatally castrated or not, however, receive supplementary testosterone, their lordosis responding is decreased (Coniglio & Clemens, 1976; Coniglio et al., 1973a; DeBold & Whalen, 1975; Gerall et al., 1975; Swanson, 1971; Tiefer & Johnson, 1975). While Coniglio and Clemens (1976) found no defeminization in Day 1 castrates given two daily injections of 100 µg testosterone, 50 µg testosterone on Days 1-10 decreased total lordosis duration. DeBold and Whalen (1975) found that as little as 5 µg testosterone propionate further defeminized intact males. These studies clearly show that defeminization results from postnatal exposure to testosterone, and that this process can be blocked by neonatal castration of males.

The time period which is maximally effective for suppressing lordosis is the first few days after birth. Coniglio and Clemens (1976) found that 100 µg testosterone on Days 1-2 or 3-4 increased lordosis frequency (defeminization). DeBold and Whalen (1975) found that when graded doses of testosterone propionate were given 24 hours after birth, 50 µg maximally inhibited lordosis while 5 and 10 µg were less effective. As little as 10 µg testosterone propionate decreased total lordosis duration on either Day 1 or 3 but not later in development. The time period for defeminization, then, is more restricted than the time period of Day 1-7 for the masculinization of females (DeBold & Whalen, 1975). Swanson (1971) found that 0-48 hours was the maximum sensitive period for defeminization, while at least 0-72 hours was maximal for masculinization.

One of the most exciting and heuristic findings of research in the development of sexual behavior has been that testosterone is not the only hormone capable of masculinizing and defeminizing behavior in hamsters. Coniglio et al. (1973a) found that testosterone, testosterone propionate, estradiol, estradiol benzoate or diethylstilbestrol induced mounting potential in Day 1 castrate male hamsters; dihydrotestosterone and androsterone, non-aromatizable androgens, were not effective. Tiefer and Johnson (1975) also found that with extended hormone treatment, from Day 1-20, 10 µg androstenedione was equivalent to 10 µg testosterone in masculinizing males. These same two hormones were effective in inducing mounting in females; for equal treatments, however, females were less masculinized in their behavior than castrated males.

Paup et al. (1972) found that the synthetic estrogen, diethylstilbestrol was actually more effective in inducing mounting in female hamsters than either testosterone or testosterone propionate, while androsterone was ineffective. Whalen and Etgen (1978) compared the ability of testosterone propionate, estradiol benzoate, and a synthetic estrogen, RU-2858, to masculinize female hamsters. Although the dose-response was not linear, as little as 500 ng estradiol benzoate or 50 pg RU-2858 induced mounting in females; the only dose of testosterone propionate used, 1 μg, also masculinized females. These studies have shown that estorgen is very potent in masculinizing behavior.

Defeminization of behavior can also be achieved by estrogens and androgens which can be converted to estrogens. Coniglio et al. (1973b) found that diethylstilbestrol decreased total lordosis duration in female hamsters, while in the same study, androsterone, a non-aromatizable androgen, and 25 μg testosterone were not effective. Gerall et al. (1975) did obtain weak suppression of lordosis in females given dihydrotestosterone or androsterone but the suppression was minimal compared to that produced by testosterone. In studying the dose response relationship of defeminization in female hamsters, Whalen and Etgen (1978) found that as little as 50 ng estradiol benzoate or 100 pg RU-2858 decreased mean lordosis duration in females; in the same experiment, 1 μg testosterone propionate did not defeminize. Thus either natural or synthetic estrogens are more potent than androgens in defeminizing females. Males are similarly affected. Coniglio et al. (1973a) found decreased total lordosis duration in male hamsters receiving either testosterone propionate, estradiol, estradiol benzoate or diethylstilbestrol but not

testosterone, dihydrotestosterone or androsterone. Gerall et al. (1975) found that silastics of testosterone implanted on Days 2-10 (with castration on Day 5) suppressed lordosis strongly; dihydrotestosterone was weak and androsterone had no effect.

Aromatization

Physiological studies have confirmed not only that aromatization is important in sexual differentiation, but have also localized the aromatization enzymes. These are localized in limbic areas which also bind steroid hormones, regulate gonadotropin release, and are involved in the neural control of sexual behavior (Naftolin, Ryan, Davies, Reddy, Flores, Petro, Kuhn, White, Takaoka & Wolin, 1975; Reddy, Naftolin & Ryan, 1974). Moreover, these enzymes are functional in the brain of the neonatal rat. When ^{3}H-testosterone is injected in neonatal male and female rates, ^{3}H-estradiol is recovered from cell nuclei in the preoptic area, hypothalamus and amygdala (Lieberburg & McEwen, 1975; Weisz & Gibbs, 1974).

Neonatal rat brains also have receptors which bind estrogen. When tritium labelled estradiol or diethylstilbestrol is injected into Day 3 rats, estrogen is recovered from cell nuclei in the preoptic area, hypothalamus, amygdala and cerebral cortex; binding capacity on Day 3 is about 1/3 the adult levels (McEwen, Plapinger, Chaptal, Gerlach & Wallach, 1975). Other studies have reported high levels of estrone and estradiol in the plasma of neonatal rats, both male and female (see Gorski, Harlan & Christensen, 1977). While the source of these estrogens is controversial, plasma levels of estrogen may be higher in the neonate than in the adult.

Estrogens normally in the plasma of postnatal female rats do not actually masculinize these females. Neonatal rat plasma and brain contain a specific estrogen binding protein, alpha-fetoprotein (Raynaud, Mercier-Bodard & Baulieu, 1971). Because of this binding protein, the estrone and estradiol found in the neonatal rat may be effectively inactivated (McEwen, Lieberburg, Maclusky & Plapinger, 1976). This protein disappears from the blood at 3 weeks of age, when the adult estrogen binding capacity increases (McEwen et al., 1975; Plapinger & McEwen, 1973). When exogenous estrogen masculinizes and defeminizes, it probably does so by overloading this protective estrogen binding system.

These studies still do not show that estrogen is the hormone that normally masculinizes and defeminizes behavior. One critical experiment is to block the aromatization of testosterone to estradiol; if masculinization and defeminization of behavior are also blocked, then aromatization is a necessary step in normal sexual differentiation. Aromatization of testosterone to estradiol is a multiple reaction process with several enzyme systems involved; the pathways for this conversion have not been worked out completely but several intermediate products have been identified. Two major reactions are first the hydroxylation of C-19 and finally conversion of the A ring to an aromatic structure (Engel, 1975).

One compound which inhibits estrogen biosynthesis from precursors in human placental microsomes and in the neonatal rat brain is ATD (1,4,6-Androstatrien-3,17-dione). Figure 2 shows a diagram of the testosterone, estradiol and ATD molecules. ATD was first tested in a placental microsome system, where it was tested for competitive

Figure 2. ATD blocks the conversion of testosterone to estradiol (figure courtesy of Brian A. Gladue).

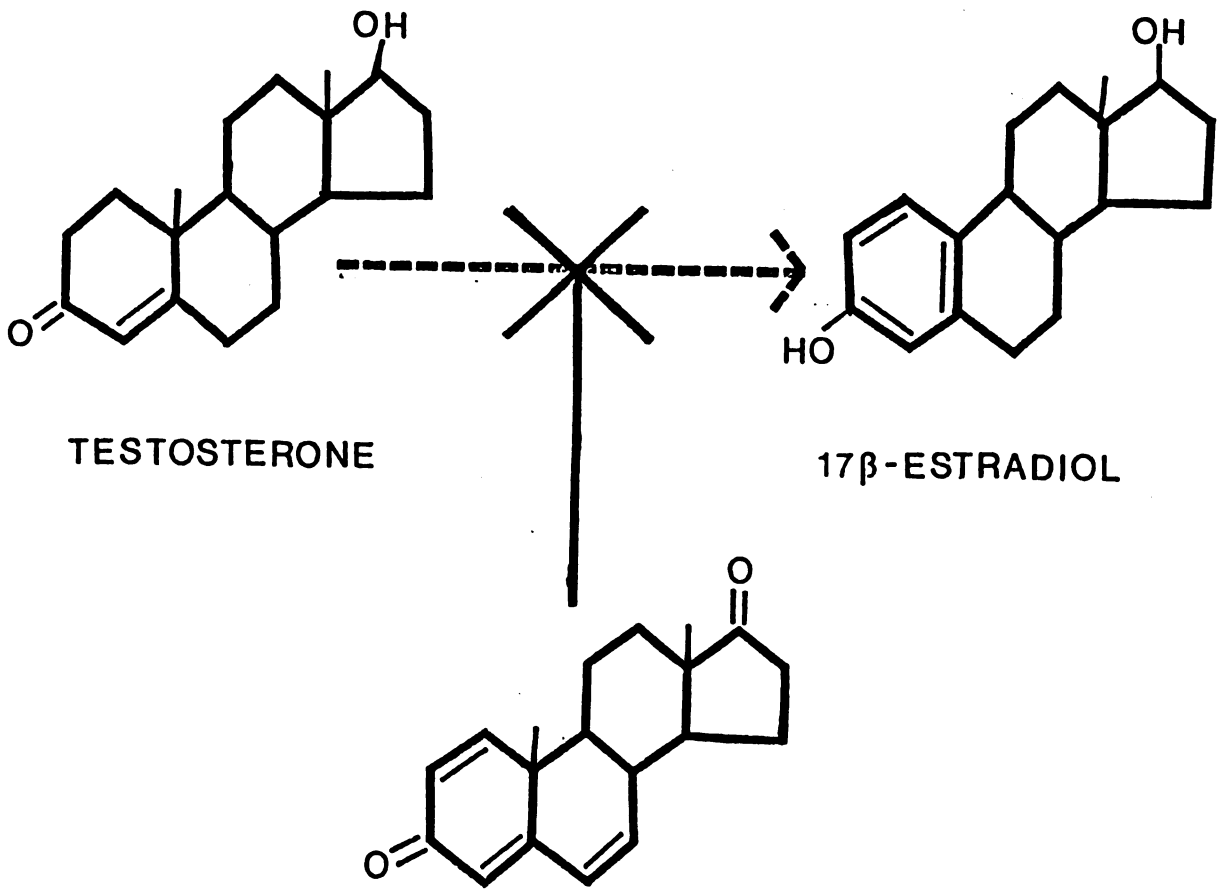
OH
O
TESTOSTERONE
OH
HO
17β-ESTRADIOL
O
O
1,4,6-ANDROSTATRIEN-3,17-DIONE (ATD)

Figure 2.

inhibition at ratios of .5/1, 1.5/1 and 3/1; percent inhibition ranged from 44 to 88%; ATD appears to inhibit hydroxylation (Schwarzel, Kruggel & Brodie, 1973). Lieberburg, Wallach and McEwen (1977) implanted Day 3 female rats with silastics of ATD and then injected ^{3}H-testosterone; the concentration of estradiol was significantly reduced in whole homogenates (from 1.40 ± 0.04 to 0.82 ± 0.06) and in cell nuclei (from 1.78 ± 0.35 to 0.32 ± 0.06) in the neonatal rat brain.

The ability of ATD to block behavioral masculinization and defeminization neonatally and its ability to interfere with the expression of adult sexual behavior has been studied in the rat. The hypothesis that testosterone is converted to estrogen before becoming behaviorally active has been supported. Christensen and Clemens (1975) infused ATD along with wither estradiol or testosterone into the preoptic area of adult castrate male rats; ATD blocked the activation of mounting by testosterone but did not block mounting induced by estradiol. Morali, Larsson and Beyer (1977) found that ATD, as well as other aromatase inhibitors, blocked the masculine sexual behavior of adult castrates given a single high dose of testosterone. Concurrent administration of estrogen (testosterone propionate + ATD + estradiol benzoate) increased the proportion of males mounting, intromitting and ejaculating. These studies provided supportive evidence for the aromatization hypothesis in the adult male rat. More recently, Gladue, Dohanich and Clemens (in press) have shown that ATD could block testosterone induced lordosis in the adult female rat, but did not impair estrogen induced lordosis; this is additional behavioral evidence that ATD can inhibit aromatization.

The ability of ATD or ADT to block testosterone induced sexual differentiation has also been tested in the rat. Booth (1977), on Days 1-5 postnatally, gave castrated males either 50 μg testosterone, 500 μg ADT (an aromatization inhibitor similar to ATD), or both. Males treated with both testosterone and ADT did not ejaculate as adults and were not defeminized. Inhibition of defeminization by ATD has been shown in other studies (McEwen, Lieberburg, Chaptal & Krey, 1977; Vreeburg, van der Vaart & van der Schoot, 1977), although Vreeburg et al. (1977) did not find that ATD interfered with masculinization. ATD can also block defeminization in the rat when given prenatally; Clemens and Gladue (in press) found male and female offspring of mothers injected with ATD showed enhanced sensitivity to estrogen and progesterone as adults. In the rat, then, behavioral masculinization or defeminization can be blocked by ATD given during perinatal life.

GENERAL METHODS

Animals

Male and female golden hamsters (Mesocricetus auratus) used in both experiments were born in the Hormones and Behavior Laboratory at Michigan State University; stimulus hamsters and breeders were obtained as adults from Charles River suppliers. All animals were maintained on a reversed day-night cycle of 14 hours light and 10 hours dark, with lights off at 1100 hours. Purina Lab Chow and tap water were available ad libitum; mothers were given sunflower seeds as a dietary supplement and were provided with nesting material. At weaning, 21 days of age, pups were housed in unisexual groups of 2-7 animals of the same age and treatment groups. Hamsters in groups of 2-4 were housed in cages with dimensions of 16.8 x 24.0 x 14.4 cm and groups of 5-7 were housed in cages of 19.2 x 40.8 x 14.4 cm.

Postnatal Treatments

On days 2-4 after birth (day of birth considered Day 1), all pups were injected subcutaneously each day with control or experimental solutions. A 26 gauge needle was used for injection under the skin on the lower back region; the fluid was then deposited at the nape of the neck. The injection site was sealed immediately with flexible collodion to prevent leakage.

For groups castrated postnatally, all males from a litter were castrated on Day 1 by using cryogenic anesthesia (ice). The testes were removed from their position in the body cavity with the aid of a dissecting microscope, and the incision along the abdomen was sutured with surgical silk. Pups were warmed on a heating pad before being returned to the nest.

General Testing Procedure

All tests for masculine and feminine sexual behavior were conducted in a dimly illuminated room between 1200-1600 hours. Large aquaria (50.8 x 27.0 x 30.5 cm) with Sanicel corncob bedding (Vivarium Research Inc.) covering the bottom were used as test arenas. An Esterline-Angus event recorder was used to record all behavioral responses during testing.

When testing for masculine sexual behavior, each experimental animal received a 3 minute period to adapt to the test arena, followed by a 10 minute test with a receptive stimulus female. Receptivity was induced in ovariectomized females by 3 daily injections of 12 μg estradiol benzoate, followed by 500 μg progesterone four hours prior to behavioral testing on the 4th day. Stimulus females were screened for sexual receptivity with sexually vigorous males just prior to behavioral testing with the experimental subjects. All mounts, intromissions and ejaculations shown by the experimental animals were recorded. When tested for feminine sexual behavior, each experimental animal was placed in a testing arena with a sexually vigorous male for 10 minutes. Each time the experimental animal showed lordosis, the time spent in lordosis was recorded.

After all the behavioral data were collected, the experimental animals were killed using an overdose of ether anesthesia. For males, body weight, ano-genital distance and the length of the penile bone and cartilage were measured. For females, ano-genital distance and body weight were measured. All data were analyzed by one or two factor analysis of variance, followed by a Student-Newman-Keuls comparison or t-tests, respectively.

EXPERIMENT 1

In Experiment 1, the conversion of testosterone to estradiol was blocked by an aromatization inhibitor, ATD (1,4,6-Androstatrien-3,17-dione). The hypotheses tested were that behavioral masculinization and defeminization induced by testostrone would be blocked in female hamsters, Day 1 castrate males and intact males by ATD, but behavioral masculinization and defeminization produced by estradiol would not be blocked by ATD.

Two goals were considered in choosing hormone doses and doses of the aromatization inhibitor, ATD, for this study: to obtain a minimum level of masculinization and defeminization, and to maximize competitive inhibition by ATD. Doses of hormone, chosen from past behavioral studies with hamsters, were 50 or 100 μg testosterone, and 2 μg estradiol; in both cases the alcohol form of the hormone was used. For ATD, two doses, 0.5 and 1.0 mg, were chosen from the literature on the potency of this aromatization inhibitor. This produced the following ratios of ATD to testosterone: 20:1 (1.0 mg ATD + 50 μg T), 10:1 (1.0 mg ATD + 100 μg T) and 5:1 (0.5 mg ATD + 100 μg T). As an additional control, ATD was combined with 2 μg estradiol to verify that ATD does not interfere with behavioral masculinization and defeminization produced by estradiol.

ATD or its control vehicle, propylene glycol, was injected twice daily, once at approximately 800 hours and again at 2000 hours. One

half of the daily dose was given at a time, dissolved in a volume of .01 cc. Estradiol, testosterone, or the control vehicle, sesame oil, was injected once daily at approximately 900 hours, one hour after the first ATD injection. The volume of hormone injected was .03 cc. Males of designated groups were castrated on Day 1. The treatment groups for females, Day 1 castrate males and intact males, along with the number of animals per treatment group (in parentheses) are indicated below:

Females (Groups = 11)

PG + 100 µg T (15)
1.0 mg ATD + 100 µg T (13)
0.5 mg ATD + 100 µg T (12)
1.0 mg ATD + oil (14)
0.5 mg ATD + oil (8)
PG + 2 µg E (14)
1.0 mg ATD + 2 µg E (10)
0.5 mg ATD + 2 µg E (10)
1.0 mg ATD + 50 µg T (12)
PG + oil (12)
PG + 50 µg T (10)

Day 1 Castrate Males (Groups = 8)

PG + 100 µg T (10)
PG + 50 µg T (13)
1.0 mg ATD + 100 µg T (9)
1.0 mg ATD + 50 µg T (11)
1.0 mg ATD + oil (7)
PG + 2 µg E (9)
1.0 mg ATD + 2 µg E (11)
PG + oil (6)

Intact Males (Groups = 9)

PG + 100 µg T (11)
1.0 mg ATD + 100 µg T (11)
0.5 mg ATD + 100 µg T (14)
1.0 mg ATD + oil (10)
0.5 mg ATD + oil (10)
PG + 2 µg E (11)
1.0 mg ATD + 2 µg E (14)
0.5 mg ATD + 2 µg E (11)
PG + oil (8)

Females were ovariectomized at 60 days, and intact males were castrated at 60 days; sexual behavior tests for males and females began at approximately 70 days. Each animal received four weekly tests for masculine sexual behavior, and four weekly tests for feminine sexual behavior; each test was 10 minutes as described in

the General Methods. A period of six weeks with no hormone treatment separated masculine and feminine behavior tests for each animal. Approximately half the animals in each group received male tests first, and half started with female tests.

Daily injections of 300 µg testosterone propionate were given starting one week before the first test for masculine sexual behavior; masculine tests were given on days 7, 14, 21 and 28 of hormone treatment. When tested for female behavior, experimental animals received three daily injections of 6 µg estradiol benzoate, followed by 500 µg progesterone on the day of testing, four hours before the test.

Results

The experimental, or independent variable, in Experiment 1 was postnatal hormone treatment, and the dependent variables were masculine and feminine sexual behavior scores. Only animals completing both masculine and feminine behavior tests were included in the data analysis. The scores for each animal on each of the four weekly behavior tests were averaged, so each animal had a single mean behavior score for feminine behavior tests, and a single mean score for each measure on masculine behavior tests. Figures 3 through 5 show the masculine behavior scores for all postnatal treatment groups.

Masculine behavior scores were analyzed differently for females and Day 1 castrate males on the one hand, and intact males on the other. For females and Day 1 castrate males, no animals ejaculated, intromissions were relatively few, and most animals either rear mounted or head and side mounted. Because of the number of zero scores

in each of the categories of masculine behavior, a mean masculine behavior score ($\bar{X}$MALE) was computed for female hamsters and Day 1 castrate males. This was the sum of all masculine behavior categories shown on the masculine behavior tests. The postnatally intact male groups showed all components of masculine behavior at reliable levels; these were analyzed separately as head and side mounts (HSM), rear mounts (RM), intromissions (I) and ejaculations (E).

Masculine Behavior - Females. The mean masculine behavior scores ($\bar{X}$MALE) for female hamsters are shown in Figure 3. A comparison of masculine behavior scores of females showed significant differences between postnatal treatments [$F(10,119) = 11.54$, $p < .0001$]. Control females receiving propylene glycol + oil were less masculinized in their behavior than females receiving any of the hormone treatments (except 1.0 mg ATD + 50 µg T); there were no differences between groups receiving 1.0 mg ATD + oil, 0.5 mg ATD + oil or 1.0 mg ATD + 50 µg T ($p > .05$). Thus, females not receiving hormones were not masculinized (controls), ATD by itself did not masculinize behavior, and 1.0 mg ATD completely blocked the masculinizing action of 50 µg T. While all other hormone treatment combinations of testosterone or estradiol masculinized behavior to some extent, the high dose of ATD (1.0 mg) partially blocked the masculinization produced by 100 µg T ($p < .05$), while the low dose of ATD (0.5 mg) did not block masculinization in females.

Masculine Behavior - Day 1 Castrate Males. The mean masculine behavior scores for Day 1 castrate males are shown in Figure 4. For Day 1 castrate males, a comparison of all groups via one way analysis of variance showed significant differences between postnatal treatments

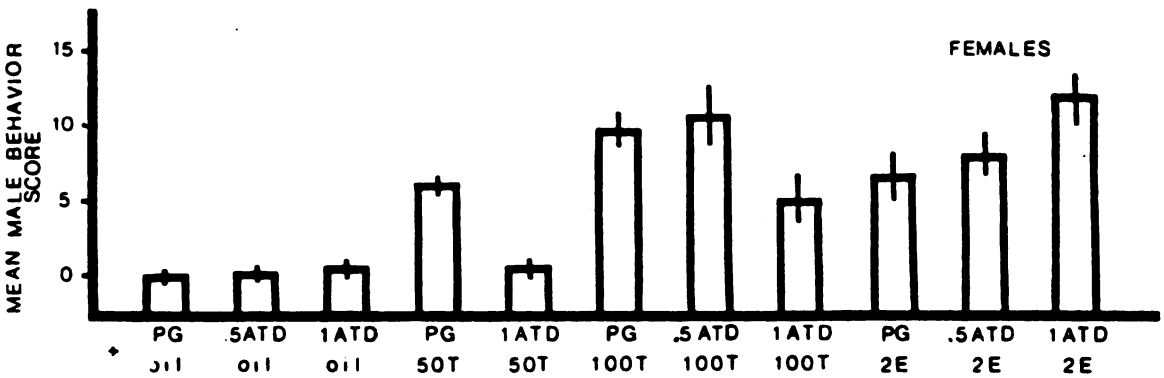

Figure 3. Mean masculine behavior scores for female postnatal treatment groups; each treatment of ATD (mg), hormone (μg) or control vehicle was given on Days 2-4 after birth.

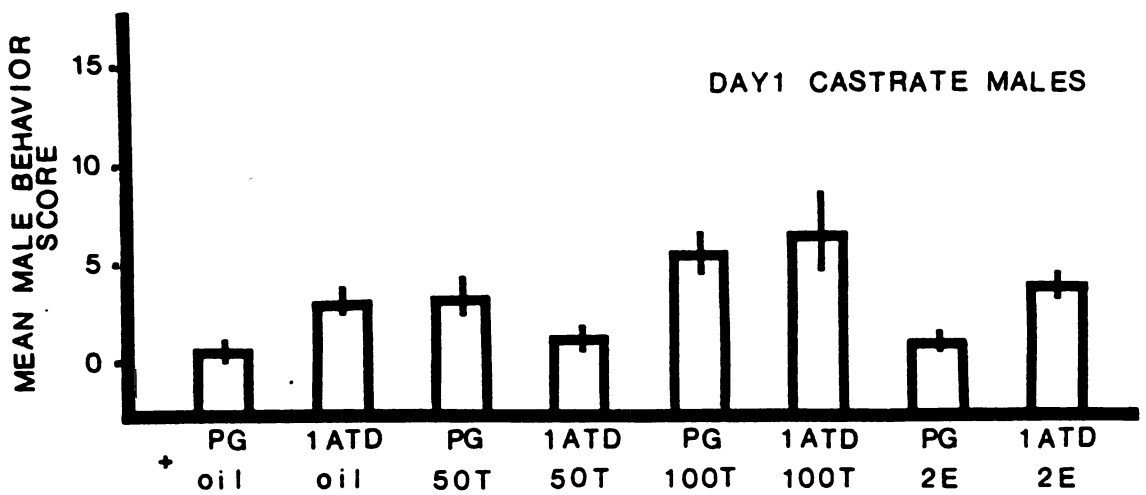

Figure 4. Mean masculine behavior scores for Day 1 castrate male postnatal treatment groups; each treatment of ATD (mg), hormone (μg) or control vehicle was given on Days 2-4 after birth.

[$F(7,68) = 4.15$, $p < .0008$]. Day 1 castrate male hamsters receiving 1.0 mg ATD + 50 μg T were not different from groups receiving no hormone ($p > .05$), while 1.0 mg ATD did not block 100 μg T, the high dose of testosterone ($p > .05$). Day 1 castrate males receiving 1.0 mg ATD + oil alone mounted more than expected, and males receiving PG + 2 μg E mounted less than expected ($p < .05$). Otherwise, these results agree with the data from the female groups.

Masculine Behavior - Intact Males. For intact males, a more detailed analysis was made of masculine behavior scores since more masculine behavior was shown. Figure 5 shows rear mounts, intromissions and ejaculations for all postnatal treatment groups. Since there was no difference for head and side mounts for any groups ($p > .05$), these data are not listed. The range of head and side mounts was 2.6 ± 0.5 (1.0 mg ATD + oil) to 5.0 ± 1.1 (PG + 100 μg T). Rear mounts did differ between postnatal treatment groups [$F(8,91) = 2.89$, $p < .006$]. Males receiving PG + 2 μg E or 1.0 mg ATD + 2 μg E showed more rear mounts than males receiving just 1.0 mg ATD + oil ($p < .05$); so, the addition of postnatal estradiol to intact males increased the number of rear mounts.

Intromission frequency also differed between postnatal treatment groups [$F(8,91)] = 2.75$, $p < .009$]. Males receiving 1.0 mg ATD + 100 μg T intromitted more than males receiving either dose of ATD + oil ($p < .05$). This shows that ATD was not able to block the combined effect of endogenous and exogenous testosterone. A similar pattern was shown for ejaculation frequency, which also differed between postnatal treatments [$F(8,91) = 2.70$, $p < .01$]. Males receiving 1.0 mg ATD + 100 μg T ejaculated more than males receiving either dose of ATD + oil ($p < .05$).

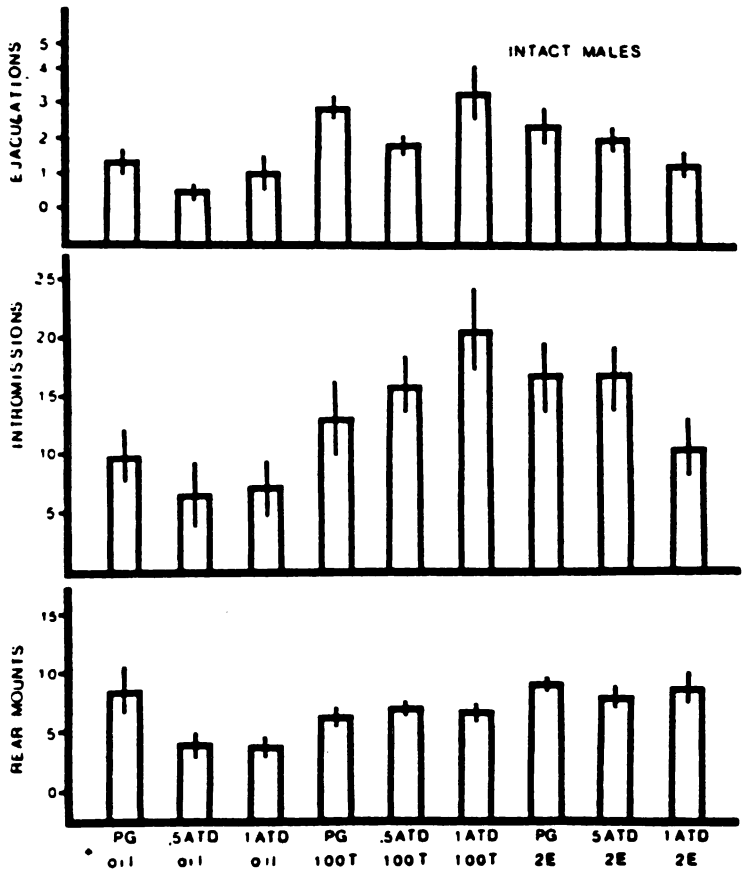

Figure 5. Rear mounts, intromissions and ejaculations for intact male postnatal treatment groups; each treatment of ATD (mg), hormone (μg) or control vehicle was given on Days 2-4 after birth.

Order of Testing Effects for Masculine Behavior. In the experimental design for Experiment 1, the test sequence of animals within each group was randomized, so approximately half would receive feminine behavior tests first, and half would receive masculine behavior tests first. Although counterbalancing was used as a control procedure, no independent effects due to order of testing were expected. In the initial analysis, all scores for animals within each postnatal treatment group were pooled, regardless of order of testing. However, while recording the data, I noticed differences within groups that seemed related to the order of testing (all behavior tests were conducted without awareness of the treatment group being tested). A two way analysis of variance was used to test for the significance of this order effect.

For masculine behavior shown by females, order of testing was a significant variable [$F(1,106) = 4.41$, $p < .038$]. This means that the masculine behavior scores of females were influenced by whether females received their four weekly masculine behavior tests first, or after feminine behavior tests were completed. Table 1 shows the mean ± standard error of masculine behavior scores for females, broken down by order of testing; the last column indicates significant differences within groups as shown by t-tests. Females receiving postnatal treatments of 1.0 mg ATD + 100 μg T had significantly higher masculine behavior scores if male tests were given first; the same was true for females receiving 1.0 mg ATD + 2 μg E postnatally.

For masculine behavior shown by Day 1 castrate males, an interaction between postnatal treatment and order of testing was significant [$F(7,60) = 2.69$, $p < .017$]. Table 2 shows the masculine behavior

Table 1. Masculine Sexual Behavior ($\bar{X}$ MALE) for Female Postnatal Treatment Groups.

Groups	All	$\bar{X}$ MALE Female Test First	Male Test First	t-tests
PG + oil n =	0.1 ± 0.1 (12)	0.0 ± 0.0 (5)	0.1 ± 0.1 (7)	
0.5 ATD + oil n =	0.2 ± 0.2 (8)	0.4 ± 0.4 (5)	0.0 ± 0.0 (3)	
1.0 ATD + oil n =	0.4 ± 0.2 (14)	0.2 ± 0.2 (11)	1.1 ± 0.6 (3)	
PG + 50T n =	7.1 ± 1.0 (10)	6.9 ± 1.6 (3)	7.1 ± 1.4 (7)	
1.0 ATD + 50T n =	0.6 ± 0.2 (12)	0.7 ± 0.4 (6)	0.5 ± 0.3 (6)	
PG + 100T n =	9.6 ± 1.5 (15)	9.4 ± 1.5 (9)	10.0 ± 3.0 (6)	
0.5 ATD + 100T n =	10.5 ± 2.1 (12)	9.8 ± 2.7 (7)	11.5 ± 3.7 (5)	
1.0 ATD + 100T n =	5.1 ± 1.5 (13)	1.7 ± 0.9 (7)	9.1 ± 2.2 (6)	$+(11) = 3.28$, $p < .007$
PG + 2E n =	6.7 ± 1.4 (14)	5.9 ± 1.4 (9)	8.3 ± 3.1 (5)	
0.5 ATD + 2E n =	8.0 ± 1.7 (10)	9.2 ± 2.7 (6)	6.1 ± 1.0 (4)	
1.0 ATD + 2E n =	11.8 ± 1.8 (10)	2.9 ± 0.6 (2)	14.0 ± 1.2 (8)	$+(8) = 4.32$, $p < .003$

Table 2. Masculine Sexual Behavior ($\bar{X}$ MALE) for Day 1 Castrate Male Postnatal Treatment Groups.

Groups	All	$\bar{X}$ MALE Female Test First	Male Test First	t-tests
PG + oil	0.6 ± 0.5	0.0 ± 0.0	1.1 ± 1.0	
n =	(6)	(3)	(3)	
1.0 ATD + oil	2.9 ± 1.3	0.5 ± 0.2	6.0 ± 1.7	+(5) = 3.95,
n =	(7)	(4)	(3)	p < .011
PG + 50T	3.2 ± 0.9	3.4 ± 1.3	3.0 ± 1.3	
n =	(13)	(7)	(6)	
1.0 ATD + 50T	1.2 ± 0.3	1.3 ± 0.5	1.1 ± 0.5	
n =	(11)	(4)	(7)	
PG + 100T	5.6 ± 1.3	3.9 ± 1.5	7.3 ± 2.1	
n =	(10)	(5)	(5)	
1.0 ATD + 100T	6.6 ± 2.0	8.1 ± 2.2	1.4 ± 1.4	
n =	(9)	(7)	(2)	
PG + 2E	1.0 ± 0.4	0.4 ± 0.4	2.1 ± 0.9	+(7) = 2.73,
n =	(9)	(6)	(3)	p < .029
1.0 ATD + 2E	4.1 ± 0.7	5.1 ± 1.1	3.2 ± 0.9	
n =	(11)	(5)	(6)	

scores of Day 1 castrate males broken down by order of testing. Masculine behavior scores were significantly higher when masculine behavior was tested before feminine behavior for males treated postnatally with PG + 2 μg E or 1.0 mg ATD + oil. Although the order of testing for 1.0 mg ATD + 100 μg T was not significant [$t(7) = 1.56$, $p < .162$], masculine behavior scores were higher when masculine behavior tests followed female behavior tests; this difference probably accounted for the significant interaction.

Again, for intact males receiving postnatal treatments, order of testing was a significant variable for rear mounts [$F(1,82) = 26.63$, $p < .0001$] as seen in Table 3; for intromissions [$F(1,82) = 92.81$, $p < .0001$] as seen in Table 4; and for ejaculations [$F(1,82) = 79.19$, $p < .0001$] as seen in Table 5. By visual inspection of Tables 3-5, and by examining the t-test results, it is obvious that there are large differences due to order of testing for intact males. Although most of these are in the direction of higher masculine behavior scores if male tests are given first, significant interaction effects for rear mounts [$F(8,82) = 2.25$, $p < .03$] and for ejaculations [$F(8,82) = 4.33$, $p < .0001$] indicate that for some postnatal treatments, the direction of difference for order effects was reversed.

Feminine Behavior - Females. Feminine behavior was analyzed in females using mean total lordosis duration ($\bar{X}$TLD) as the behavioral measure; this is the total lordosis duration divided by lordosis frequency. Figure 6 shows the mean ± the standard error for feminine behavior scores for all postnatal treatment groups. A significant difference was found between treatment groups in a one way comparison [$F(10,119) = 5.82$, $p < .0001$]. Further tests revealed that females

Table 3. Rear Mounts for Intact Male Postnatal Treatment Groups

Groups	Rear Mounts All	Rear Mounts Female Test First	Rear Mounts Male Test First	t-tests
PG + oil n =	8.6 ± 1.9 (8)	5.0 ± 0.9 (5)	14.7 ± 0.6 (3)	+(6) = 7.74, p < .0001
0.5 ATD + oil n =	4.1 ± 1.0 (10)	4.5 ± 1.1 (6)	3.6 ± 2.0 (4)	
1.0 ATD + oil n =	3.8 ± 0.9 (10)	2.4 ± 0.9 (6)	5.9 ± 1.5 (4)	
PG + 100T n =	6.2 ± 1.1 (11)	4.5 ± 1.2 (7)	9.2 ± 1.5 (4)	+(9) = 2.43, p < .038
0.5 ATD + 100T n =	7.0 ± 0.9 (14)	5.5 ± 1.5 (6)	8.2 ± 1.1 (8)	
1.0 ATD + 100T n =	6.5 ± 0.8 (11)	5.4 ± 1.3 (5)	7.4 ± 1.0 (6)	
PG + 2E n =	8.9 ± 0.8 (11)	8.6 ± 0.7 (6)	9.2 ± 1.5 (5)	
0.5 ATD + 2E n =	8.0 ± 1.1 (11)	6.2 ± 0.8 (3)	8.7 ± 1.5 (8)	
1.0 ATD + 2E n =	8.7 ± 1.3 (14)	5.4 ± 1.8 (6)	11.2 ± 1.1 (8)	+(12) = 2.86, p < .014

Table 4. Intromissions for Intact Male Postnatal Treatment Groups

Groups	Intromissions All	Intromissions Female Test First	Intromissions Male Test First	t-tests
PG + oil n =	9.8 ± 2.2 (8)	5.8 ± 1.7 (5)	16.4 ± 1.8 (3)	+(6) = 4.06, p < .007
0.5 ATD + oil n =	6.5 ± 2.7 (10)	5.1 ± 2.5 (6)	8.7 ± 6.2 (4)	
1.0 ATD + oil n =	7.1 ± 2.6 (10)	3.6 ± 2.1 (6)	12.4 ± 4.8 (4)	
PG + 100T n =	13.0 ± 3.2 (11)	7.1 ± 2.3 (7)	23.2 ± 4.5 (4)	+(9) = 3.58, p < .005
0.5 ATD + 100T n =	15.8 ± 2.5 (14)	10.3 ± 3.7 (6)	19.8 ± 2.7 (8)	
1.0 ATD + 100T n =	20.4 ± 3.7 (11)	8.8 ± 2.6 (5)	30.0 ± 2.2 (6)	+(9) = 6.29, p < .0001
PG + 2E n =	16.7 ± 3.0 (11)	8.8 ± 1.7 (6)	26.0 ± 2.3 (5)	+(9) = 6.30, p < .0001
0.5 ATD + 2E n =	16.7 ± 2.4 (11)	5.8 ± 0.9 (3)	20.8 ± 1.7 (8)	+(9) = 5.17, p < .001
1.0 ATD + 2E n =	10.5 ± 2.3 (14)	3.2 ± 1.5 (6)	16.1 ± 2.4 (8)	+(12) = 4.16, p < .001

Table 5. Ejaculations for Intact Male Postnatal Treatment Groups.

Groups	Ejaculations All	Female Test First	Male Test First	t-tests
PG + oil n =	1.4 ± 0.4 (9)	0.7 ± 0.2 (5)	2.6 ± 0.4 (3)	+(6) = 4.34, p < .005
0.5 ATD + oil n =	0.5 ± 0.3 (10)	0.6 ± 0.4 (6)	0.5 ± 0.5 (4)	
1.0 ATD + oil n =	1.0 ± 0.5 (10)	0.4 ± 0.3 (6)	1.8 ± 1.0 (4)	
PG + 100T n =	2.8 ± 0.3 (11)	0.9 ± 0.4 (7)	3.1 ± 0.9 (4)	+(9) = 2.48, p < .035
0.5 ATD + 100T n =	1.8 ± 0.4 (14)	1.5 ± 0.5 (6)	2.1 ± 0.6 (8)	
1.0 ATD + 100T n =	3.3 ± 0.8 (11)	0.8 ± 0.4 (5)	5.4 ± 0.4 (6)	+(9) = 8.66, p < .0001
PG + 2E n =	2.4 ± 0.6 (11)	0.8 ± 0.2 (6)	4.2 ± 0.6 (5)	+(9) = 5.79, p < .0001
0.5 ATD + 2E n =	2.0 ± 0.4 (11)	0.2 ± 0.2 (3)	2.6 ± 0.4 (8)	+(9) = 3.96, p < .003
1.0 ATD + 2E n =	1.2 ± 0.4 (14)	0.0 ± 0.0 (6)	2.0 ± 0.5 (8)	+(12) = 3.93, p < .002

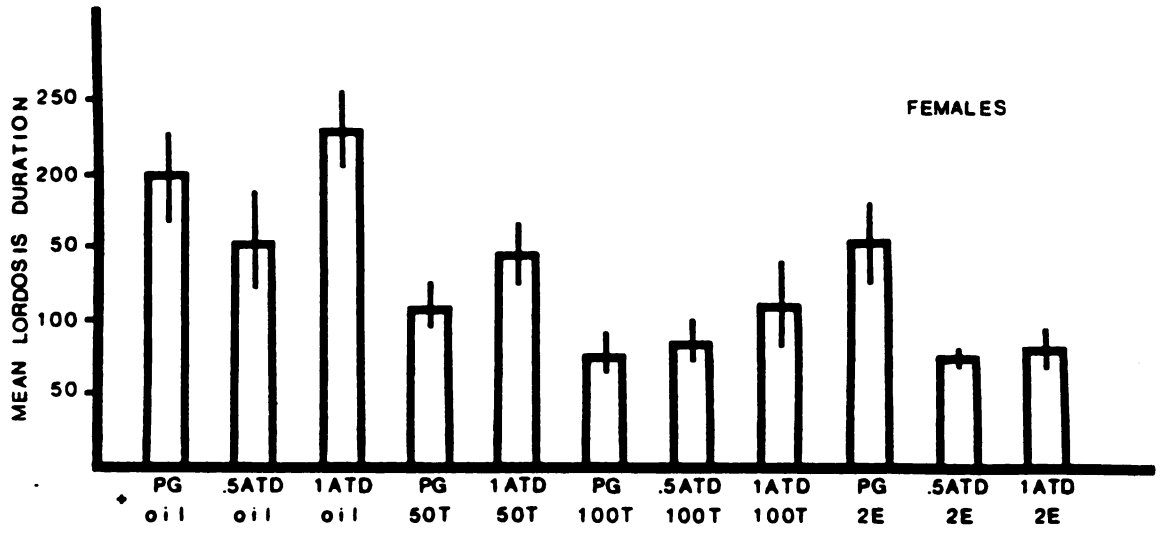

Figure 6. Mean lordosis durations for female postnatal treatment groups; each treatment of ATD (mg), hormone (μg) or control vehicle was given on Days 2-4 after birth.

receiving 1.0 mg ATD + oil or PG + oil showed longer mean lordosis durations than the most defeminized groups: PG + 100 μg T, 0.5 ATD + 100 μg T, 1.0 mg ATD + 2 μg E and 0.5 mg ATD + 2 μg E ($p < .05$). Although 0.5 mg ATD did not block the defeminization produced by 100 μg T, a higher dose of ATD (1.0 mg), or lower dose of testosterone (50 μg), produced less defeminization (no statistical tests).

Order of testing was a significant variable in a two way analysis of variance [$F(1,106) = 32.29$, $p < .0001$] and interaction effects were also significant [$F(10,106) = 2.95$, $p < .003$]. Table 6 shows the feminine behavior scores for females, broken down by order of testing; the last column indicates significant differences between groups as shown by t-tests. Females receiving postnatal treatments of PG + 100 μg T, 1.0 mg ATD + 100 μg T, PG + 2 μg E or PG + oil showed significantly longer mean total lordosis durations if feminine behavior tests were preceded by masculine behavior tests.

Feminine Behavior - Males. Neither male group, Day 1 castrate males or intact males, differed between postnatal treatments on mean total lordosis duration. Figure 7 shows feminine behavior scores for Day 1 castrate males and Figure 8 shows feminine behavior scores for postnatally intact males. Male hamsters did show shorter mean lordosis durations than females, including Day 1 castrates receiving no additional hormone treatments. Possibly, the doses of hormone used in this experiment were too low to further defeminize males.

Morphological Data. At the time of sacrifice, body weights and ano-genital distance measurements were obtained for animals in all postnatal treatment groups; in addition, the length of the penile bone and cartilage was measured in males. Testes weights were

Table 6. Feminine Sexual Behavior ($\bar{X}$ TLD) for Female Postnatal Treatment Groups.

Groups	All	$\bar{X}$ TLD Female Test First	$\bar{X}$ TLD Male Test First	t-tests
PG + oil	199.8 ± 27.7	114.5 ± 17.0	260.7 ± 28.0	+(10) = 4.02, p < .002
n =	(12)	(5)	(7)	
0.5 ATD + oil	153.4 ± 34.6	114.0 ± 29.4	219.2 ± 69.5	
n =	(8)	(5)	(3)	
1.0 ATD + oil	231.5 ± 24.8	238.0 ± 27.8	207.7 ± 64.4	
n =	(14)	(11)	(3)	
PG + 50T	108.9 ± 14.6	85.8 ± 30.8	118.8 ± 16.3	
n =	(10)	(3)	(7)	
1.0 ATD + 50T	144.3 ± 20.0	128.1 ± 29.0	160.4 ± 28.4	
n =	(12)	(6)	(6)	
PG + 100T	74.8 ± 14.0	49.8 ± 15.4	112.1 ± 18.6	+(13) = 2.57, p < .023
n =	(15)	(9)	(6)	
0.5 ATD + 100T	83.6 ± 15.3	73.4 ± 25.3	98.0 ± 10.9	
n =	(12)	(7)	(5)	
1.0 ATD + 100T	110.5 ± 29.9	54.9 ± 10.9	175.4 ± 54.0	+(11) = 2.36, p < .038
n =	(13)	(7)	(6)	
PG + 2E	154.1 ± 27.7	87.1 ± 15.0	274.7 ± 22.7	+(12) = 7.17, p < .0001
n =	(14)	(9)	(5)	
0.5 ATD + 2E	75.0 ± 5.6	75.2 ± 6.5	74.6 ± 11.4	
n =	(10)	(6)	(4)	
1.0 ATD + 2E	81.2 ± 13.0	62.6 ± 30.9	85.8 ± 15.0	
n =	(10)	(2)	(8)	

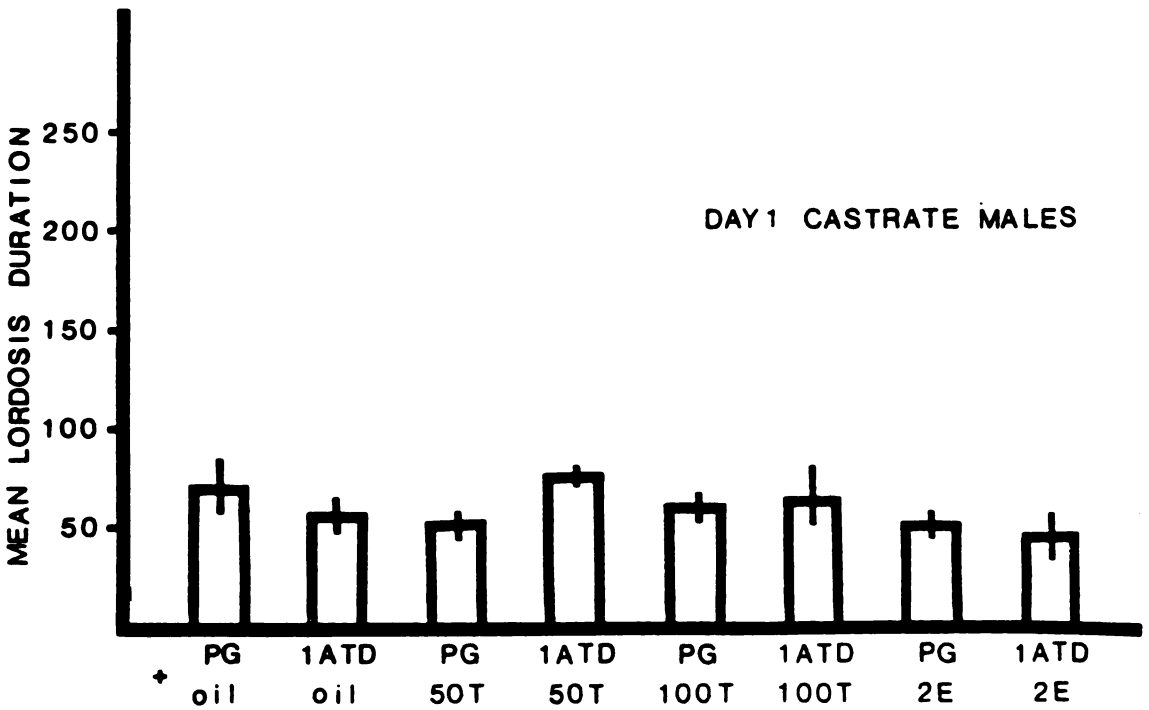

Figure 7. Mean lordosis durations for Day 1 castrate male postnatal treatment groups; each treatment of ATD (mg), hormone (μg) or control vehicle was given on Days 2-4 after birth.

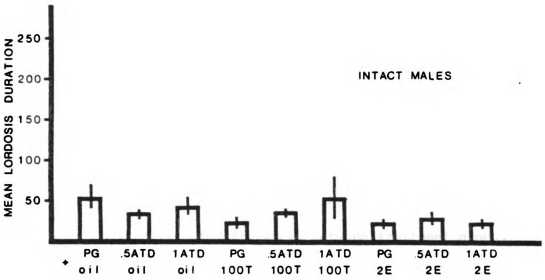

Figure 8. Mean lordosis durations for intact male postnatal treatment groups; each treatment of ATD (mg), hormone (μg) or control vehicle was given on Days 2-4 after birth.

obtained from intact males at the time of adult castration (Day 60). Body weights for all animals are shown in Table 7 and morphological measurements are shown in Table 8.

For body weight in females, there was a significant difference between postnatal treatment groups [$F(10,117) = 4.705$, $p < .0001$]. Females receiving either PG + 100 μg T, 1.0 mg ATD + oil or PG + 2 μg E weighed more than females receiving PG + 50 μg T or 1.0 mg ATD + 50 μg T postnatally ($p < .05$). There were no differences in body weights for Day 1 castrate males. For intact male postnatal treatment groups, body weight did differ [$F(8,91) = 2.066$, $p < .0472$]. Males receiving PG + 100 μg T postnatally weighed more than males receiving 1.0 mg ATD + 2 μg E ($p < .05$).

For females in all postnatal treatment groups, the ano-genital distance was shorter than for any male postnatal treatment group; there were no significant differences between treatment groups. Ano-genital distance did differ for Day 1 castrate males [$F(7,68) = 4.807$, $p < .0002$] and for intact male postnatal treatment groups [$F(8,91) = 2.482$, $p < .0176$]. For Day 1 castrate males, males receiving PG + 100 μg T postnatally had a longer ano-genital distance than males receiving either PG + 50 μg T, 1.0 mg ATD + 50 μg T, 1.0 mg ATD + 2 μg E or PG + oil ($p < .05$). For intact males, males receiving either 0.5 mg ATD + oil or PG + 2 μg E postnatally had longer ano-genital distances than males receiving 1.0 mg ATD + 2 μg E ($p < .05$).

For males, when the length of the penile bone and cartilage was measured, there were significant differences between postnatal treatment groups for Day 1 castrate males [$F(7,68) = 6.846$, $p < .0001$] and

Table 7. Body Weight (gm) for All Postnatal Treatment Groups.

Groups	Females	Day 1 Castrate Males	Intact Males
PG + oil	126.4 ± 4.5	114.9 ± 5.2	134.5 ± 4.7
0.5 ATD + oil	118.1 ± 4.9	---	141.5 ± 6.1
1.0 ATD + oil	139.6 ± 5.3	118.9 ± 4.4	132.3 ± 3.1
PG + 50T	114.7 ± 3.1	119.5 ± 2.5	---
1.0 ATD + 50T	113.5 ± 4.0	126.8 ± 3.9	---
PG + 100T	141.0 ± 5.7	128.7 ± 3.9	144.5 ± 9.8
0.5 ATD + 100T	132.5 ± 3.9	---	132.1 ± 4.3
1.0 ATD + 100T	130.5 ± 2.8	133.2 ± 4.5	127.2 ± 5.5
PG + 2E	142.4 ± 4.7	130.3 ± 4.2	136.0 ± 0.6
0.5 ATD + 2E	130.6 ± 6.1	---	140.7 ± 4.2
1.0 ATD + 2E	133.5 ± 4.2	127.1 ± 5.4	120.9 ± 3.5

Table 8. Morphological Measures for All Postnatal Treatment Groups: Anogenital Distance (A-G), Penile Bone and Cartilage Length (PB & C) and Testes Weight (Testes Wt).

Groups	Females	Day 1 Castrate Males		Intact Males		
	A-G (mm)	A-G (mm)	PB & C (mm)	A-G (mm)	PB & C (mm)	Testes Wt (gm)
PG + oil	10.5 ± 0.3	13.8 ± 0.7	4.4 ± 0.3	15.6 ± 0.7	5.1 ± 0.1	---
0.5 ATD + oil	9.9 ± 0.4	---	---	16.3 ± 0.4	5.4 ± 0.1	3.8 ± 0.1
1.0 ATD + oil	10.8 ± 0.2	15.4 ± 0.6	4.8 ± 0.2	15.6 ± 0.3	5.4 ± 0.1	3.3 ± 0.2
PG + 50T	10.8 ± 0.5	13.8 ± 0.3	4.7 ± 0.1	---	---	---
1.0 ATD + 50T	10.3 ± 0.2	14.1 ± 0.4	4.2 ± 0.2	___	___	___
PG + 100T	10.2 ± 0.3	16.5 ± 0.7	4.6 ± 0.1	15.3 ± 0.5	5.7 ± 0.1	2.8 ± 0.3
0.5 ATD + 100T	10.7 ± 0.3	---	---	15.8 ± 0.4	5.5 ± 0.1	2.7 ± 0.3
1.0 ATD + 100T	10.4 ± 0.2	15.7 ± 0.4	4.3 ± 0.1	15.5 ± 0.4	5.5 ± 0.1	3.5 ± 0.2
PG + 2E	9.9 ± 0.3	14.9 ± 0.4	3.5 ± 0.2	16.5 ± 0.6	6.0 ± 0.1	3.3 ± 0.3
0.5 ATD + 2E	10.1 ± 0.3	---	---	15.0 ± 0.3	5.4 ± 0.2	3.5 ± 0.1
1.0 ATD + 2E	10.6 ± 0.3	13.8 ± 0.4	4.2 ± 0.1	14.3 ± 0.3	5.4 ± 0.1	2.9 ± 0.3

for intact males [$F(8,81) = 4.099$, $p < .0003$]. For Day 1 castrate males, the length of the penile bone and cartilage for males treated postnatally with PG + 2 μg E was significantly less than the length for all other treatment groups ($p < .05$). For intact male postnatal treatment groups, the length of the penile bone and cartilage was significantly shorter for males receiving PG + oil postnatally than the length for males receiving PG + 100 μg T or PG + 2 μg E ($p < .05$).

The final morphological measure was adult testes weight in postnatally intact male treatment groups. For one group, males treated with PG + oil, testes weights were not available for comparison. For the other postnatal treatment groups, there was a significant difference in testes weights [$F(7,83) = 2.576$, $p < .0188$]. Males treated postnatally with 0.5 mg ATD + oil had significantly greater testes weights than males treated postnatally with 0.5 mg ATD + 100 μg T ($p < .05$).

EXPERIMENT 2

Although the neural events associated with defeminization are unknown, one result of exposure to gonadal hormones during early life in rats is a reduced sensitivity to estrogen and progesterone. While male rats and androgenized females both show female sexual behavior in response to estrogen and progesterone, they are less sensitive to equivalent doses of ovarian hormones, i.e., their feminine sexual behavior scores are low. Since estrogen and progesterone normally synergize to facilitate receptivity, defeminization could involve a change in estrogen sensitivity, progesterone sensitivity, or both. Although male rats and females given androgen during development are less sensitive to estrogen, i.e., higher doses are needed for longer time periods (Kow, Malsbury & Pfaff, 1974), the major effect of exposure to postnatal gonadal hormones seems to be a lack of response to the facilitatory effects of progesterone (Clemens, 1972).

Several studies have compared the female behavior of adult male and female hamsters in response to acute estrogen only, chronic estrogen only or estrogen and progesterone combined. With acute exposure to estrogen only (1 or 2 injections), feminine sexual behavior in the hamster is very sporadic; Carter et al. (1973) reported that neither male nor female hamsters showed lordosis with acute estrogen only. While Tiefer (1970) found no difference in total lordosis

duration between male and female hamsters receiving acute estrogen, 90% of the males but only 22% of the females showed some lordosis. Clemens, Hiroi and Gorski (1969) also found increased receptivity in femlae rats given testosterone propionate postnatally and treated with estrogen only as adults. The data are not clear on how estrogen sensitivity is affected by postnatal hormones.

Chronic treatment with estrogen (more than 3 days) induced long durations of lordosis in both male and female hamsters (Carter et al., 1973; Johnson, 1975; Noble & Alsum, 1975; Tiefer & Johnson, 1971). In comparing the lordosis of adult male and female hamsters given very high doses of estrogen (200 μg EB/day), Johnson (1975) found that males who had been castrated on Day 1 had higher lordosis scores than adult castrates, while females given testosterone during development had lower lordosis scores than normal females. With only 6 μg EB/day of adult hormone treatment, however, the presence or absence of postnatal testosterone did not influence responsiveness to estrogen (Johnson, 1975). Carter et al. (1973) did report that lordosis scores with chronic estrogen were higher for females.

Progesterone facilitates female sexual behavior in both male and female hamsters, but feminine behavior scores are lower in males (Johnson, 1975; Tiefer, 1970). Carter et al. (1973), however, determined that although the total scores for males were lower, facilitation by progesterone was of equal magnitude in males and females. Again, although Tiefer and Johnson (1971) found that the amount of facilitation to progesterone depended on the amount of estrogen priming (6 μg EB vs. 100 μg EB), Carter et al. (1973) did not find

that the amount of estrogen (6.6 µg EB bs. 666 µg EB) altered the response to progesterone.

It is not clear from comparing these studies why the discrepancies exist. Although a comparison is being made of changes in sensitivity to ovarian hormones, data are not available on threshold doses of estrogen and progesterone which could be used as a basis for evaluating defeminization. In the present study, female hamsters receiving a range of postnatal hormone treatments were tested for female behavior in response to low doses of estrogen only, or a low does of estrogen plus varying amounts of progesterone. The aim of this experiment was to determine if any of the postnatal treatments which defeminized also altered either estrogen or progesterone sensitivity.

Female hamsters (Groups = 9) used in this experiment received the following postnatal treatments:

PG +	100 µg T	(10)
1.0 mg ATD +	100 µg T	(6)
0.5 mg ATD +	100 µg T	(6)
1.0 mg ATD +	oil	(8)
0.5 mg ATD +	oil	(8)
PG +	2 µg E	(6)
1.0 mg ATD +	2 µg E	(7)
0.5 mg ATD +	2 µg E	(8)
normal females		(7)

The injection procedures were identical to those in Experiment 1. The normal female controls were purchased from Charles River. All females were ovariectomized as adults and tested synchronously. One week after ovariectomy, females were given an injection series of 6 µg estradiol benzoate for three days, followed by 400 µg progesterone on the 4th day. This same injection schedule was given a second week followed by a 10 minute mating test with a stimulus male. Thereafter, all females were

tested weekly according to the hormone schedule listed below. All doses of estradiol benzoate were given for three days, and all progesterone doses were given on the 4th day, four hours before testing:

Week	Treatment
1	0 µg EB
2	1.5 µg EB
3	3 µg EB
4	6 µg EB
5	12 µg EB
6	3 µg EB + 50 µg P
7	3 µg EB + 100 µg P
8	3 µg EB + 200 µg P
9	3 µg EB + 400 µg P

Results

Feminine behavior scores for female hamsters exposed to various postnatal hormone treatments were compared for mean total lordosis duration ($\bar{X}$TLD). With adult treatment of 0 to 12 µg estradiol benzoate only, females did not consistently show feminine sexual behavior with any of the estrogen doses. For the few females who did show lordosis, usually on one test only, the responses were similar to those seen in other studies: very brief. This experiment shows that neither normal female hamsters, in agreement with other studies, nor defeminized females, were behaviorally responsive to acute estrogen only.

In the second half of the experiment, varying doses of progesterone were combined with a priming dose of 3 µg estradiol benzoate. Table 9 shows the mean ± the standard error for the postnatal treatment groups at each dose of progesterone. Because of the within group variance, a square root transformation of the data was performed, prior to one way analysis of variance for each of the progesterone

Table 9. Feminine Sexual Behavior ($\bar{X}$ TLD) for Female Postnatal Treatment Groups Tested with Varying Doses of Progesterone (P).

Groups	N	50 µg P	100 µg P	200 µg P	400 µg P
Normal	7	346.4 ± 99.7	292.8 ± 95.2	149.4 ± 69.6	363.7 ± 79.8
0.5 ATD + oil	8	87.8 ± 29.5	108.1 ± 24.2	107.9 ± 26.0	306.9 ± 80.7
1.0 ATD + oil	8	162.9 ± 49.8	326.1 ± 91.1	274.6 ± 80.4	132.5 ± 16.8
PG + 100T	10	94.3 ± 27.3	212.8 ± 65.2	125.0 ± 29.4	120.3 ± 25.0
0.5 ATD + 100T	6	109.1 ± 42.4	130.0 ± 23.6	115.7 ± 30.2	179.5 ± 82.6
1.0 ATD + 100T	6	14.6 ± 10.8	131.7 ± 39.9	106.5 ± 36.1	141.1 ± 16.6
PG + 2E	6	104.9 ± 89.6	108.2 ± 38.5	176.3 ± 58.2	167.6 ± 55.2
0.5 ATD + 2E	8	179.8 ± 91.6	241.8 ± 82.0	160.2 ± 61.3	188.2 ± 37.8
1.0 ATD + 2E	7	58.8 ± 34.5	141.6 ± 76.0	229.5 ± 90.8	187.7 ± 58.2

doses (50, 100, 200 and 400 μg progesterone). Females from all postnatal treatment groups showed a definite facilitation of feminine sexual behavior in response to progesterone combined with a priming dose of estradiol benzoate. However, there were no differences between postnatal treatment groups; facilitation was equal for all groups at all dose levels of progesterone.

Ano-genital distance did not differ for any postnatal treatment groups, but there was a significant difference in body weights [$F(8,57) = 2.5664$, $p < .0183$]. Normal females weighed more than females receiving 0.5 mg ATD + oil postnatally ($p < .05$). The means and standard errors for these morphological measures are shown in Table 10.

Table 10. Morphological Measures for Female Postnatal Treatment Groups Tested for Estrogen and Progesterone Sensitivity.

Groups	Body Weight (gm)	Anogenital Distance (mm)
Normal	145.1 ± 8.8	10.8 ± 0.4
0.5 ATD + oil	117.0 ± 5.3	10.0 ± 0.2
1.0 ATD + oil	131.2 ± 4.9	10.7 ± 0.3
PG + 100T	121.3 ± 2.6	9.6 ± 0.1
0.5 ATD + 100T	121.2 ± 2.6	9.2 ± 0.2
1.0 ATD + 100T	117.8 ± 5.4	8.8 ± 0.2
PG + 2E	112.7 ± 6.4	10.1 ± 0.5
0.5 ATD + 2E	133.8 ± 4.7	10.0 ± 0.4
1.0 ATD + 2E	133.9 ± 8.8	9.9 ± 0.4

DISCUSSION

This present study extends the concept of the aromatization hypothesis by showing that inhibition of aromatization blocked behavioral masculinization and defeminization in the hamster. This is in agreement with previous work by Coniglio, Paup and Clemens (Coniglio et al., 1973a,b; Paup et al., 1972, 1974) which provided a data base and suggestion for this interpretation of hormone action during early life. This is further evidence that for the hamster, at least, estrogen is the major active hormone involved in behavioral sexual differentiation. The effects of inhibition of aromatization on the two behavioral processes examined in this study, masculinization and defeminization will be considered separately.

Masculinization

In the present study, both testosterone and estradiol induced the potential for adult mounting in females and Day 1 castrate males given hormones on Days 2-4 after birth. Although the testosterone doses were 50 or 25 times greater than the estradiol dose, the effects on induction of mounting were equivalent. This corresponds to an estimated in vivo aromatization rate of 1-2% for testosterone conversion to estradiol (Naftolin et al., 1975). The degree of masculinization produced by either hormone treatment was, however, minimal; most animals mounted but did not intromit or ejaculate. With higher

hormone doses, a more extended time period of administration, or with the propionate or benzoate form of these hormones, more complete masculinization of behavior probably would have resulted.

The 20:1 ratio of ATD to testosterone (1.0 mg ATD + 50 μg T) completely blocked behavioral masculinization in females and Day 1 castrate male hamsters, while the 10:1 ratio (1.0 mg ATD + 100 μg T) only achieved a partial block of masculinization in females; the 10:1 ratio did not block masculinization in Day 1 castrate males. The 5:1 ratio (0.5 mg ATD + 100 μg T) did not inhibit masculinization for either group. In addition, ATD had no independent or synergistic effects on behavior; animals receiving ATD alone were not masculinized and ATD had no effect on masculinization produced by estradiol. These results conform to the idea that aromatization of testosterone to estradiol is essential for behavioral masculinization.

Intact males were also tested for inhibition of behavioral masculinization by ATD. Those males which received ATD showed fewest intromissions and ejaculations. The highest scores for intromissions and ejaculations were obtained by males treated postnatally with 1.0 mg ATD + 100 μg T or PG + 100 μg T. With a combined exposure to endogenous and exogenous testosterone, the amount of ATD present was not sufficient to influence behavior.

Defeminization

For feminine sexual behavior, the mean total lordosis duration ($\bar{X}$TLD) was compared for the various postnatal treatment groups. This behavioral measure incorporates two aspects of feminine behavior which are altered in the hamster by postnatal exposure to gonadal steroids:

the total lordosis duration which decreases as a result of exposure to gonadal steroids, and the frequency of lordosis, which increases as a result of early steroid exposure. In brief, defeminized females show short lordosis responses, hence the frequency is increased. For female hamsters in the present experiment, control animals not receiving hormones showed the longest mean duration of lordosis. Feminine behavior in female hamsters was suppressed by estradiol, testosterone and the 5:1 ratio of ATD to testosterone (0.5 mg ATD + 100 μg T). The 20:1 ratio (1.0 mg ATD + 50 μg T) partially blocked the defeminizing effects of testosterone. ATD had no independent effects on behavior.

All male groups, regardless of postnatal treatment, were defeminized in comparison to females, but there were no differences in feminine behavior between the postnatal treatment groups. The doses of hormone used in the present study may have been insufficient to further defeminize the behavior of males. Since, for Day 1 castrate males, development occurs in the absence of postnatal gonadal hormones, these males should be equivalent to normal females in their lordosis responding. However, other studies have shown that Day 1 castrate male hamsters are not equivalent to normal females. Coniglio et al. (1973a,b) found a lordosis duration of 418 seconds for females but only 108 seconds for males castrated on Day 1. These authors suggested several possible explanations for the difference.

First, since the maximum sensitive period for defeminization is within the first 3 days after birth, perinatal exposure to testicular androgen prior to castration may be sufficient to defeminize. Second, the stimulus male may react differently to a male partner than to a

female partner. Kow et al. (1976), using manual stimulation, found a greater degree of receptivity in males than is usually found in mating tests. Third, vaginal stimulation, which is not available to the male, may influence lordosis duration; in female hamsters at least, prolonged genital stimulation can be a signal to terminate lordosis (Carter et al., 1976).

Order of Testing Effects

In the present study, all animals were tested for both masculine and feminine sexual behavior. Since no data are available on whether adult exposure to particular hormones (either testosterone or estrogen + progesterone) or whether exposure to a particular testing situation (masculine or feminine behavior) can affect subsequent sexual behavior, order of testing was counterbalanced in this experiment. An exact 50:50 ratio was not maintained for all postnatal treatment groups, in part because of the logistics of housing and testing animals, and in part because of animal mortality.

When the data were analyzed for order of testing effects, significant differences were found within postnatal treatment groups for some female and Day 1 castrate male treatment groups, and for most intact male treatment groups. These differences generally meant that animals which had received masculine behavior tests before feminine behavior tests had higher masculine behavior scores; for female treatment groups, females from some postnatal treatment groups had higher feminine scores if feminine sexual behavior was tested last. Several interpretations of these findings are possible.

There are three major differences for sequence of testing: proximity to time of castration, prior exogenous hormone exposure and prior behavioral testing. For intact males castrated as adults, different time intervals between castration and testing for masculine behavior could account for the order effects. That is, males receiving masculine behavior tests first had a 10 day period with no hormones between castration and the initiation of testosterone propionate replacement. For males tested in the reverse sequence, 6 weeks with no hormone treatment preceded masculine behavior tests. Since other behavioral studies have shown that with increasing time following castration, either increasing amount of hormones or longer exposure to hormones is needed to reinstate behavior (Christensen, Coniglio, Paup & Clemens, 1973), males tested after 6 weeks of hormonal deprivation might be expected to show reduced masculine behavior.

For masculine and feminine behavior tests in female hamsters, the interpretation used for intact males is not applicable. Although order of testing effects were not as pervasive across postnatal treatment groups in female hamsters, when order effects were seen, both masculine and feminine sexual behavior were facilitated if masculine behavior tests occurred first. For females, it is possible that there were some carryover effects from ovarian secretions which could facilitate the display of mounting. Several studies have shown that masculinized female hamsters mount prior to ovariectomy, and continue to mount for short periods following ovariectomy (Carter et al., 1972; Swanson & Crossley, 197; Tiefer & Johnson, 1975). Residual effects of ovarian secretions could possibly account for increased mounting in some female groups when masculine behavior was tested first.

The finding that, for some postnatal treatment groups, feminine behavior scores were higher in female hamsters when tested following masculine behavior tests, is counter-intuitive. Beach (1976) has applied a deprivation desensitivity interpretation, used to explain decrements in post-castration masculine behavior, to explain a similar phenomenon for feminine behavior. For female rats, females are less sensitive to estrogen and progesterone with increasing time post-ovariectomy. Since the order of testing effects seen for feminine behavior in this experiment were opposite to this, another explanation is needed. Day 1 castrate males also showed order of testing effects for masculine behavior for several postnatal treatment groups, with higher masculine behavior scores for masculine behavior tests first. Since these males were deprived of hormones from Day 1 after birth until Day 70 when adult hormone treatments began, it is difficult to see how deprivation desensitivity could account for order of testing effects in these males.

Of the three major differences for sequence of testing, proximity to time of gonadectomy offers a plausible explanation for order of testing effects for masculine behavior in postnatally intact male treatment groups and for masculine behavior in female treatment groups. For masculine behavior in Day 1 castrate males and for feminine behavior in females, though, the effects of first hormone treatment and first behavioral testing upon the second test sequence must be considered. There are no data available to suggest that performing adult masculine sexual behavior will alter an animal's ability to show feminine responses, or vice versa. However, adult hormone treatments could possibly modify the effects of perinatal hormone treatments.

Two examples will illustrate the possibility that adult hormone treatment could modify the process of sexual differentiation. Noble (1974, 1977) found that normal female hamsters will mount receptive females when given prolonged (4 week) very high doses of gonadal hormones as adults; he suggested that such adult hormone exposure might modify the female's ability to respond to hormones. Gorski has also described a physiological syndrome in female rats, the delayed anovulatory syndrome, where it is suspected that feedback from adult ovarian hormones can modify the pattern of gonadotropin release (Gorski et al., 1977). Female rats which are lightly masculinized by postnatal testosterone begin cycling at puberty but then become anovulatory. Failure to ovulate, then, is initiated by postnatal exposure to testosterone and then completed by postpubertal ovarian feedback. There could be an analogous behavioral syndrome where adult testosterone treatment or adult estrogen + progesterone treatment could be completing or modifying the process of sexual differentiation begun at birth.

Estrogen and Progesterone Sensitivity

Defeminization, i.e., decreased lordosis scores in response to estrogen and progesterone, may result from altered sensitivity to ovarian hormones (Clemens, 1972; Kow et al., 1974). One way to determine the mechanism of behavioral defeminization is to look for changes in responsiveness to estrogen and progesterone in the adult. In Experiment 2, female hamsters receiving testosterone or estradiol during early life, with or without ATD, were tested for lordosis in response to varying doses of estrogen alone and in response to estrogen

and varying doses of progesterone. With acute doses of estradiol benzoate, ranging from 1.5 to 12 µg, females did not show measurable levels of feminine behavior. This finding is consistent with previous work showing that female hamsters are not responsive to estrogen alone, and does not indicate that postnatal hormone treatment altered sensitivity to estrogen.

After completion of the estrogen only lordosis testing, females from all groups were tested for lordosis with 3 µg estradiol benzoate combined with progesterone in doses ranging from 50 to 400 µg. Progesterone facilitated lordosis for all postnatal treatments, but there was no difference between groups in response to any dose of progesterone. Facilitation was equal for all treatment groups, whereas a dose response relationship had been anticipated. DeBold, Martin and Whalen (1976) found that for normal female hamsters, 50 µg progesterone was the minimal dose to facilitate lordosis, while 200 µg progesterone produced maximal responding. The priming dose of estrogen was different for the two studies: DeBold et al. (1976) gave a single injection of 10 µg estradiol benzoate, while in the present study, 3 daily injections of 3 µg estradiol benzoate preceded progesterone injections. The differences in estrogen priming, however, probably were not sufficient to account for the behavioral difference. The female hamsters in this experiment appeared to be more sensitive to progesterone, and this was not altered by postnatal treatment.

In Experiment 2, lordosis scores were quite variable. This can be seen in the means and standard errors for each dose of progesterone (as shown in Table 9), and also in the lack of consistent response for each postnatal treatment group with increasing doses of

progesterone. Two periods of hormone exposure need consideration in interpreting this variability: postnatal treatment and adult hormone treatment. In Experiment 1 of this study, females were defeminized by the same doses of testosterone and estradiol used in this second experiment. Failure to find differences between groups, then, is most likely due to differences in adult hormone treatment.

Before being tested for lordosis in response to estradiol benzoate plus varying doses of progesterone, females from all treatment groups were exposed to 4 weeks of hormone treatment and testing with estradiol benzoate only (3 injections per week). It is possible that this estrogen priming, although not affecting behavior at the time of testing, could have attenuated or left incomplete the process of defeminization. The variability in behavior, then, would reflect the variability in this adult action of low doses of estradiol benzoate. Again, the analogy can be made to the adult action of hormones on the mounting of normal female hamsters (Noble, 1974, 1977) and on the delayed anovulatory syndrome in the female rat (Gorski et al., 1977). This present experiment, though, does not provide support for the hypothesis that defeminization in the hamster involves changes in sensitivity to either estrogen or progesterone. Furthermore, since the initiation of this study, it has been found that estrogen influences the amount of progesterone receptors available (Feder, Landau, Marrone & Walker, 1977). If so, then any altered response to progesterone would be difficult to interpret, since it might reflect either decreased responsiveness to progesterone or an alteration in the capacity of estrogens to induce progesterone receptors.

Morphological Measures

For the morphological measures recorded in this study, one difference was clear and consistent: females from any postnatal treatment groups had a shorter ano-genital distance than any of the male treatment groups. This morphological characteristic distinguishes between males and females. For the other morphological measures, body weight, ano-genital distance (between postnatal treatment groups) and penile bone and cartilage length (males only), no clear and consistent differences were found, although some statistically significant differences were found. The magnitude of these differences was probably not sufficient to be functionally important. Previous developmental studies in the hamster have not found a correlation between behavioral masculinization and morphological masculinization for different postnatal treatment groups (Coniglio et al., 1973a,b; Paup et al., 1972).

Conclusion

This study provided experimental support for the aromatization hypothesis; ATD, which inhibits aromatization, blocked behavioral masculinization in female hamsters, Day 1 castrate males and postnatally intact males. ATD also partially blocked defeminization in female hamsters. These data also suggest that adult hormone treatment may modify the effects of exposure to hormones in early life; further experiments are needed to test the possible nature of this modification. Finally, defeminization in the female hamster did not involve altered sensitivity to estrogen or progesterone in adulthood.

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