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Exogenous androgen during development alters adult partner preference and mating behavior in gonadally intact male rats

C.L. Henley *, A.A. Nunez, L.G. Clemens

Department of Zoology, Michigan State University, East Lansing, MI 48824, USA
Department of Psychology, Michigan State University, East Lansing, MI 48824, USA
The Neuroscience Program, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

In the rat, neonatal administration of testosterone propionate to a castrated male causes masculinization of behavior. However, if an intact male is treated neonatally with testosterone (hyper-androgen condition), male sexual behavior in adulthood is disrupted. There is a possibility that the hyper-androgen treatment is suppressing male sexual behavior by altering the male's partner preference and thereby reducing his motivation to approach the female. If so, this would suggest that exposure to supra-physiological levels of androgen during development may result in the development of male-oriented partner preference in the male. To test this idea, male rats were treated either postnatally or prenatally with testosterone, and partner preference and sexual behavior were examined in adulthood. The principal finding of this study was that increased levels of testosterone during early postnatal life, but not prenatal, decreased male sexual behavior and increased the amount of time a male spent with a stimulus male, without affecting the amount of time spent with a stimulus female during partner preference tests. Thus, the reduction in male sexual behavior produced by early exposure to high levels of testosterone is not likely due to a reduction in the male's motivation to approach a receptive female.

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Introduction

In male rats, as in many other mammalian species, exposure to endogenous testosterone during perinatal development causes masculinization, the enhancement of neural systems that mediate male-typical responses, and defeminization, the suppression of neural systems that mediate female-typical responses (Adkins-Regan, 1988; Bakker, 2003; Baum, 1979). These effects of testosterone during early development are often referred to as "organizational effects" and can occur both pre- and postnatally.

Prenatally, male rats show a testosterone surge that starts on gestation day 16 and lasts through gestation day 20 (Ward et al., 2003). This surge has been shown to be important for the differentiation of sexual behavior (Hoepfner and Ward, 1988). Male offspring of mothers treated with an anti-androgen during gestation show feminized behavior in adulthood (Neumann et al., 1966). Females that develop between two males *in utero* show more male-like sexual behavior in adulthood than females located between two females, and this effect is blocked by prenatal treatment with an anti-androgen (Clemens et al., 1978). Finally, females treated with testosterone during both the pre- and postnatal period show a greater increase in the expression of male-like sexual behavior as adults than after postnatal androgen treatment alone (Pollak and Sachs, 1975; Ward, 1969).

Castration at birth eliminates or greatly reduces the expression of male sexual behavior in adulthood (Beach and Holz, 1946). Normal masculine behavior is reinstated, however, if testosterone is replaced immediately following castration (Beach et al., 1969). In addition, female rats treated neonatally with testosterone show, in some cases, an increase in male-typical responses (Mullins and Levine, 1968; Whalen and Edwards, 1967), and males treated neonatally with the anti-androgen, flutamide, show decreases in the expression of male sexual behavior (Hernandez-Tristan and Cerezo, 2000). These findings support the idea that both pre- and postnatal endogenous testosterone mediate behavioral sex differentiation in rats.

Although testosterone is necessary for normal development of male sexual behavior, exogenous perinatal testosterone treatment of an intact male results in disruption of normal male sexual behavior (Diamond et al., 1973; Piacsek and Hostetter, 1984; Pollak and Sachs, 1975; Zadina et al., 1979). One factor that could mediate this effect of testosterone exposure is the possibility that the hyper-androgen treatment is reducing the male's motivation to approach the female. Support for this idea can be found in both the animal and human literature. Although not statistically significant, male ferrets treated

* Corresponding author. 2201 Biomedical Physical Sciences, Michigan State University, East Lansing, MI 48824, USA. Fax: +1 517 432 2789.
E-mail address: mcgove14@msu.edu (C.L. Henley).

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with testosterone perinatally appear less likely to approach a stimulus female than a stimulus male compared to control males (Baum et al., 1990). Prenatal hyper-androgen exposure has also been suggested to play a role in sexual orientation in humans. Some homosexual men have been shown to have larger genitalia (Bogaert and Hershberger, 1999), more masculine auditory evoked potentials (McFadden and Champlin, 2000), and more masculine 2D:4D digit length ratios (Rahman, 2005; Rahman and Wilson, 2003; Robinson and Manning, 2000) than heterosexual men (but see Berenbaum et al., 2009). All of these measures appear to be androgen-dependent during development, indicating that some homosexual men may be exposed to a higher level of androgen during development compared to heterosexual men.

Whereas several studies (Diamond et al., 1973; Piacsek and Hostetter, 1984; Pollak and Sachs, 1975; Zadina et al., 1979) have focused on the consummatory aspects of male sexual behavior after early exposure to elevated levels of testosterone, the current study expands these findings to include appetitive aspects of male sexual behavior including measures of partner preference. A male's motivation to approach a partner can be measured in a preference test by examining the amount of time spent with each stimulus animal. We hypothesize that the reduction in male sexual behavior after early testosterone treatment is due, at least in part, to a decrease in the male's attraction to the female. In the present study, the effect of exogenous testosterone on adult behavior was tested by treating intact male rats during either early postnatal development or prenatally with testosterone propionate and examining their adult partner preference and sexual behavior. The effects of early postnatal exposure to testosterone on serum testosterone and estradiol levels were also evaluated.

Methods

Overview of experiments

Three experiments were conducted in this study. In Experiment 1, the experimental males were exposed to early postnatal hormone treatments and stimulus males were untreated. In Experiment 2, experimental males were exposed to early postnatal hormone treatments and stimulus males were treated with the aromatase inhibitor 1,4,6 androstatriene-3,17-dione (ATD) during the early postnatal period as explained below. In Experiment 3, experimental males were exposed to prenatal hormone treatments and stimulus males were untreated.

Animals

Experimental males (Experiments 1–3)

Time-mated pregnant Long–Evans rats (Charles River, Raleigh, NC) were housed individually in plastic cages (45.5×24×21 cm) with *ad lib* food and water (Experiment 1: TP $n=10$, C $n=7$; Experiment 2: TP $n=10$, C $n=11$; Experiment 3: TP $n=5$, Oil $n=7$). The dams were kept in a 14:10-h light dark cycle with lights on at 01:00. Thirty one-inch paper towel strips were given to the dams for nest building material on gestation day (GD) 20. A subset of the male offspring of these dams became the experimental males of this study. Separate cohorts of pregnant dams were used for each experiment. In Experiments 1 and 2, on the day of birth (postnatal day [PND] 0), the litter was reduced to four male and four female pups to keep litter size uniform across groups. For litter reductions, the anogenital distance (AGD) for each pup was measured. The animals with the four shortest and the four longest measurements were retained to provide a mixed-gender litter since the AGD is shorter in females than in males. In Experiment 3, since prenatal testosterone increases AGD of both sexes, the seven animals with the largest AGD were kept to maximize the chance of picking males, and the animal with the smallest AGD was kept in an attempt to prevent forming an all-male litter. Thus, the experimental males used in the present study were not selected randomly at birth. The AGD is an indication of androgenic effects perinatally, and therefore the males used were selected on the basis of high androgen responsiveness.

Experimental male hormone treatments (Experiments 1,2)

Silastic capsules (Dow Corning; inner diameter 1.47 mm; outer diameter 1.96 mm; length 5 mm) were used to administer either testosterone propionate (Sigma; TP) or cholesterol (Sigma; C) treatments starting on the day of birth. The pups were anesthetized using ice and the capsules were implanted subcutaneously (s.c.; one treatment per litter) through a small incision on the back of the animal. These animals are referred to as TP or C males, respectively. The incision was closed using superglue (Loctite). The pups were kept under a heat lamp for approximately 1 h until the incision was dry. They were then placed back with their mothers. The implants were removed through an incision made near one end of the capsule on PND 21 under isoflurane anesthesia (Isoflo, Abbot Laboratories). First Aid Cream (Johnson and Johnson) was used on the incision, which was then closed with an Auto Clip (Clay Adams). The pups were weaned at this time and housed with same-sex littermates. Although the testosterone exposure is longer than other studies examining the effect of postnatal hyper-androgen exposure on behavior (Diamond et al., 1973; Piacsek and Hostetter, 1984; Pollak and Sachs, 1975; Zadina et al., 1979), previous studies from this laboratory show that treatment with steroid hormones or endocrine disrupting agents during this postnatal time period results in changes in the expression of adult behavior (Cummings et al., 2008; Henley et al., 2009). Two male pups randomly selected from each litter, and therefore, exposed to the same treatment, were used in these experiments. Behavioral tests began after the animals reached 90 days of age (Experiment 1: TP $n=18$, C $n=14$; Experiment 2: TP $n=20$, C $n=21$).

Experimental male hormone treatments (Experiment 3)

Pregnant dams were treated with either TP in sesame oil (2 mg/0.1 ml/day) or oil vehicle on GD 16–20. Pups were weaned on PND 21 and housed with same-sex littermates until 90 days of age when behavioral tests were conducted. These males are referred to as TP and Oil males, respectively. Two male pups from each litter were randomly selected for this experiment, and behavioral tests began after the animals reached 90 days of age (TP $n=9$, C $n=13$).

Stimulus females (Experiments 1–3)

Stimulus females were sexually experienced, gonadally intact, adult Long Evans female rats at least 60 days old (Charles River, Raleigh, NC). Prior to testing, a Silastic capsule containing 25% estradiol benzoate (Sigma) to cholesterol mixture was implanted s.c. on the back of the females while under isolurane anesthesia. The incision was closed with an Auto Clip and covered with First Aid Cream. After 4 weeks, the capsules were removed and reimplanted via a new incision in the neck. This was to prevent a loss of efficacy due to connective tissue growth around the capsule (personal observation). Stimulus females were injected s.c. with 0.5 mg progesterone 4 h prior to partner preference and sexual behavior testing.

Stimulus males (Experiments 1,3)

Sexually experienced, gonadally intact, adult Long Evans male rats at least 90 days old (Charles River, Raleigh, NC) were used as stimulus animals for the behavioral tests.

Stimulus males (Experiment 2)

The stimulus males in Experiment 2 received ATD (Sigma) postnatally. Male neonates were treated with Silastic capsules containing ATD on PND 0 through 21. Procedures were the same as those used for the early postnatal TP and C treatments. This treatment