Chapter 2
Prenatal Steroid Hormones and Sex Differences in Juvenile Rhesus Macaque Behavior

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Abstract Rhesus monkeys (Macaca mulatta) have been the primary primate model for investigating hormonal organization of juvenile sexually dimorphic behavior, primarily rough play and mounting. Large doses of androgens administered to pregnant females for 75 or more days of gestation masculinized the genitalia and juvenile behavior of female offspring. Unlike in rodents, estrogenic metabolites of androgens do not appear to play a role in behavioral sexual differentiation of rhesus monkeys as the nonaromatizable androgen, 5α-dihydrotestosterone, produced comparable behavioral masculinization in this species. Because prenatal androgen treatments masculinized both behavior and genitalia, some argued that the behavioral changes seen in androgenized rhesus monkey females reflect socialized responses to their genital's male-like appearance. By varying the timing of prenatal androgen exposure, the effects of androgens on genitals and behavior were separated. Thirty-five-day androgen treatments in early gestation masculinized female genitalia and mounting in rhesus monkeys, but did not masculinize rough play. By contrast, treatments late in gestation did not masculinize genitalia, but masculinized both rough play and mounting, thus separating genital effects of androgens from behavioral effects. Subsequent work with androgens and antiandrogens identified late gestation as a time when behavioral systems are particularly sensitive to androgens. A study of monkey's preference for human sex-typed toys found sex differences remarkably similar to those reported in children. Since the sex-typed nature of the toys would be unknown to the monkeys, the preference likely reflects a sex-difference in the predisposition for activities facilitated by the toys. Sexually differentiated behavior ultimately reflects both hormonally organized behavioral predispositions and the social experience that converts these predispositions into behavior.

Keywords Aromatization · Androgens · Masculinization · Maternal interest · Monkey · Mounting · Neonatal · Play · Prenatal · Sexual differentiation · Social behavior · Toy preference

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There is great interest in human sex differences and gender development, but experiments to identify causal mechanisms are generally impossible to do in humans. Instead researchers have turned to animal models with the rhesus monkey being the primary nonhuman primate model for investigating behavioral sex differences that may be relevant to humans. Many factors make them a valuable model species. Rhesus monkeys, like humans, have a long developmental life span, live in complex social groups, and exhibit striking sexually differentiated behavioral patterns both during development and in adulthood. Additionally, rhesus monkeys share important biological systems with humans, including a prenatal period of sexual differentiation, making them ideal for investigating basic mechanisms of hormonal effects on sexual differentiation that cannot be experimentally investigated in humans.

Sexual differentiation of behavior has been investigated in few of the many nonhuman primate species. The vast majority of studies use rhesus monkeys. While these studies have elucidated a great deal about sexual differentiation in a nonhuman primate, we know little, if anything, about the extent or the mechanisms of sexual differentiation of behavior in apes, new world primates, or non-Macaque species. However, the range of treatments investigated in rhesus monkeys and the diverse social conditions employed have revealed a number of important relationships that help frame research in other primate species, including humans. Thus this review focuses on hormonal influences on sexual differentiation of behavior in rhesus monkeys. Hormonal mechanisms of sexual differentiation in rhesus monkeys have been investigated in the context of a long history of studies on the role that hormones play in sexual differentiation.

The organizational hypothesis, the notion that androgens or their metabolites alter the developing nervous system during specific developmental periods permanently inducing behavioral characteristics of males and females, has become a central tenet of behavioral neuroendocrinology since the pioneering study of Phoenix et al. (1959). While specific details of hormonally induced organization continue to be debated (Arnold & Breedlove, 1985; Fitch & Denenberg, 1998), there is little doubt that exposure to steroid hormones, particularly androgens, during periods of developmental sensitivity permanently alters the responsivity of individuals to their environment.

Most studies of the organizational effects of steroids on the sexual differentiation of behavior has come from studies of altricial species, such as rats, mice, and hamsters, who are born prior to complete neural differentiation (Wallen & Baum, 2002), guinea pigs and Macaques being the only precocial mammalian species whose sexual differentiation has been extensively studied. Whether the distinction of altricial and precocial mammals (Gaillard et al., 1997) explains differences in the species-specific processes of sexual differentiation remains unresolved. There is little doubt, however, that precocial species differ from the more typically studied altricial species in the timing of sexual differentiation and in the role of estrogenic metabolites of androgens in sexual differentiation (Wallen & Baum, 2002). This is of particular importance for considerations of human sexual differentiation, as the

commonly used altricial rat and mouse models of sexual differentiation may not apply to precocial humans as they appear to use different hormonal mechanisms to produce behavioral sexual differentiation than is the case in precocial species. Thus the results of studies of the precocial rhesus monkey are likely to be more directly relevant to humans.

2.1 Sexually Differentiated Behavior in Rhesus Monkeys

Rhesus monkeys are born with their genitals and internal reproductive anatomy completely differentiated. Unlike altricial species, such as rats, hamsters, and mice, morphological sexual differentiation occurs prenatally in rhesus monkeys as it does in humans. Rhesus monkeys have an approximately 168-day gestation with three approximate 55-day trimesters. In males the testes differentiate in the first trimester between gestation day 38–40 (Resko, 1985). Fetal testes become steroidogenically active around gestation day 40 and secrete androgens throughout gestation with peak levels at gestation days 40–75 (second trimester), then decline with another apparent increase around gestation day 140 (third trimester) (Resko, 1985). Throughout the prenatal period males experience significantly higher levels of testosterone (T) than do females, though there are no apparent differences between the sexes in either 5α-dihydrotestosterone (DHT) or androstenedione (Resko, 1985; Resko & Ellinwood, 1981). The fetal ovaries are apparently quiescent at this time since females show significantly elevated luteinizing hormone (LH) levels in comparison to males and LH levels are suppressed by exogenous T in fetally ovariectomized females (Ellinwood et al., 1982). Thus fetal males are exposed to elevated levels of T from their own testes and females are exposed to lower, but quantifiable, T levels, presumably of maternal origin since the fetal ovary is inactive. It is not known if humans show the same pattern as multiple sampling of prenatal hormones is not possible in humans. From the reproductive anatomical difference between boys and girls it is clear at some point prenatally, likely the second and third trimester, boys experience higher level of androgens than do females.

As is the case in humans, rhesus monkeys have a period of infant and juvenile dependency and development, when behavioral predispositions fully develop. Rhesus monkeys develop more quickly than do humans (e.g., developmentally, a rhesus monkey year equals approximately four human years). Thus, while sharing many important features with humans, rhesus monkeys make a practical and valuable model system for investigating behavioral sexual differentiation.

Rhesus monkeys display several sexually differentiated patterns of juvenile and adult behavior (Lovejoy & Wallen, 1988; Wallen, 1996). The primary sexually differentiated behavioral patterns are higher levels of juvenile mounting and high energy expenditure play (rough play) in males (Goy & Wallen, 1979; Lovejoy & Wallen, 1988), greater interest in infants (Herman et al., 2003), and greater association with adult females (Lovejoy & Wallen, 1988; Wallen, 1996) in juvenile

females. Of these there are analogous human sex differences in play and interest in infants. In addition, infant distress vocalizations, which occur when infants are temporarily rejected or restrained by their mother, are also sexually differentiated in rhesus monkeys (Tomaszycki et al., 2001). Juvenile rhesus monkeys, similar to children, show social sex segregation (Hassett et al., 2010) and human-like sex differences in toy preferences (Hassett et al., 2008). In adult rhesus monkeys, play rarely occurs, but adult males display greater levels of mounting, now accompanied by intromissions and ejaculations, whereas females show increased interest in infants (Maestripieri & Wallen, 1995) and higher levels of sexual initiation (Maestripieri & Wallen, 1995; Wallen et al., 1984).

2.2 Social Influences on Behavioral Sex Differences

Social context and rearing conditions affect the expression of rhesus monkey infant and juvenile behavioral sex differences. A previous review of social influences on sexually differentiated behavior in rhesus monkeys (Wallen, 1996) concluded that some patterns of juvenile behavior differ between males and females almost completely as a result of social context and rearing history. In this regard, some sex differences in rhesus monkeys appear to be the result of socialization as is often invoked in explaining the ontogeny of human sex differences. In contrast, other behaviors are significantly affected by prenatal hormonal conditions and appear to differ between the sexes under all social and rearing conditions studied (Wallen, 1996). The present review focuses on those behavioral patterns where sex differences occur in more than one social environment, or, where the effect of socialization processes has not been investigated. Other behavioral patterns, such as juvenile aggression and submission, suggested to be sexually differentiated (Harlow & Harlow, 1962; Harlow, 1962), are now known to vary with the social environment with sex differences occurring in some environments and not in others (Wallen, 1996). The determining factor appears to be the amount of social interaction juveniles have with each other, with sex differences in juvenile rhesus monkey aggression and submission occurring only in social contexts that severely limit the amount of juvenile social interaction (Wallen, 1996).

2.3 Neonatal Hormonal Secretions and Rhesus Monkey Behavioral Sex Differences

In male rhesus monkeys, testicular activity falls on the day of birth and then increases within 24 hr., remaining at adult-like levels for the first 2–3 months of life (Mann et al., 1993; Mann et al., 1984). There does not appear to be similar neonatal ovarian activation in female rhesus monkeys, although there may be a

gonadal negative feedback suppression of female gonadotropin secretion neonatally as neonatal ovariectomy results in elevated gonadotropins (Plant, 1986). Suppressing male neonatal T secretion appears to influence the timing of puberty (Mann et al., 1993; Mann et al., 1998), but has no striking effects on either juvenile (Wallen et al., 1995) or adult male behavior (Eisler et al., 1993). Neither neonatal castration (Goy & McEwen, 1980; Pomerantz et al., 1986) nor suppression of neonatal T secretion using gonadotropin releasing hormone (GnRH) agonists or antagonists in males have produced evidence that neonatal hormonal secretions are involved in behavioral masculinization or defeminization in males or are involved in normal female sexual differentiation (Nevison et al., 1997; Brown & Dixson, 1999; Wallen et al., 1995; Wallen, 1996). The only behavioral effect of neonatal T was found when males were exposed neonatally to supraphysiological levels of T (Wallen et al., 1995); these males initiated proximity with their mothers significantly less than did either females or males whose neonatal T had been suppressed (Wallen et al., 1995). However, even though supraphysiological T levels appeared to alter juvenile male maternal independence, the effect was limited to supraphysiological levels of T, as suppressing endogenous neonatal T did not significantly alter maternal independence in males in comparison to either normal males or females (Wallen et al., 1995). This finding suggests that some aspects of juvenile social behavior may be sensitive to neonatal hormonal influences, but the effects are not striking or pervasive. It seems more likely that hormonal influences during the neonatal period elaborate predispositions that are hormonally organized prenatally. In this regard rhesus monkeys, like humans, are quite different from altricial mammals, where sex differences in adult behavior develop following elevated neonatal androgens (Corbier et al., 1992). Human male's testes, like those of monkey males, secrete nearly adult levels of T for 3–5 months neonatally (Forest, 1979; Forest & Cathiard, 1975), and this elevated T secretion is not evident in boys with congenital hypogonadotropic hypogonadism (Bouvattier et al., 2011). It is not known, however, whether the failure to experience a neonatal rise in T has any behavioral effects on human males because this condition also results in failure of increased prenatal T, resulting in extensive lack of genital masculinization by the time birth occurs (Bouvattier et al., 2011). Thus, it would not be possible to attribute any behavioral differences solely to differences in neonatal T.

2.4 Sex Differences in Maternal Treatment of Infants

Sex differences in juvenile and adult behavior could result from differential maternal treatment of male and female infants, resulting in differential developmental patterns. While the notion that sex differences in rhesus monkey social behavior stem from differences in maternal socialization is attractive, there are few data to support this notion. Rhesus monkey mothers have not been found to react differently to males and females in regard to time spent grooming, restraining, or interacting with infants of each sex (Lovejoy & Wallen, 1988; Wallen, 1996). There are, however,

two patterns of maternal behavior that may be differentially expressed to male and female infants. Goy and colleagues reported that mothers inspect the genitals of their male offspring more frequently than they do those of female offspring (Goy et al., 1988). The original report was obtained from 4–6 monkey mother-infant groups in relatively sparse surroundings that limited activities; thus the behavior might have reflected a response to limited activities available in the social environment. However, this maternal difference was also seen in larger (20–125 monkeys), more socially complex groups housed in outdoor compounds offering many behavioral opportunities (Wallen et al., 1995). In this latter case, males had either suppressed, typical, or supraphysiological neonatal T levels and maternal inspection of their male offspring's genitals was proportional to penis size (Wallen et al., 1995) and may thus reflect that the male's penis presents the opportunity for maternal manipulation not seen in females.

The only other maternal behavior expressed differentially to male and female infants is maternal responsiveness to infant distress vocalizations. Mothers more reliably retrieved male infants when the males performed distress vocalizations (Tomaszycki et al., 2001). Given that greater inspection of male infant genitals seems to be the only consistent maternal sex difference in infant treatment, it seems unlikely that juvenile behavioral sex differences described in the following sections stem from differential maternal socialization. It is more likely that they result from behavioral predispositions that reflect hormonal modulation of nervous system development.

2.5 Prenatal Hormonal Influences on Behavioral Sex Differences

Prenatal hormonal influences on behavioral differentiation have been investigated primarily by exposing genetic female fetuses to supraphysiological levels of prenatal androgens, by injecting their mothers with 5–25 mg/day of either esterified testosterone (testosterone enanthate, propionate, or cypionate) or dihydrotestosterone (dihydrotestosterone propionate) (Goy & McEwen, 1980). Altering male's prenatal hormonal environment is considerably more difficult because the hormones they are exposed to come from the secretions of their own testes, which would have to be suppressed to alter the prenatal hormonal environment, whereas in females the hormones can be exogenously administered and researchers don't have to regulate the activity of the female's ovaries. One study of Japanese macaques, a species closely related to rhesus monkeys, employed abdominally implanted silastic packets containing crystalline testosterone, which produced maternal T levels (~75 ng/ml) comparable to those produced by injections (Eaton et al., 1990). Exogenous androgen treatments typically resulted in supraphysiological maternal androgen levels, but only 1/8th to 1/10th to the elevated maternal androgens reached the fetus resulting in fetal androgen levels within the normal fetal-male range (Resko et al., 1987). Thus a

T treatment that produces 75–125 ng/ml of T in the mother will produce levels within the normal fetal range for males, but levels 10–20-fold higher than normal in fetal females (Resko et al., 1980; Resko et al., 1987). If treatment is started early enough in gestation, these amounts will completely masculinize female genitalia. The behavioral effects of such treatments are thought to reveal the processes involved in normal masculine sexual differentiation. This conclusion is based on the assumption that the undifferentiated fetus is essentially female regardless of its actual genetic constituency and that in XY individuals masculine characteristics are imposed upon essentially female primordia. By varying the timing of the prenatal treatment the effects of androgens on genital anatomy can be separated from some of their effects on sexually differentiated behavior (Goy et al., 1988). In general, androgen treatments starting around 35 days of gestation (end of the first trimester) and continuing through gestation day 75 masculinize both reproductive anatomy and juvenile mounting, but not rough play (Table 2.1).

Treatments starting after gestation day 100 (end of the second trimester) have no detectable effect on female reproductive anatomy, but masculinize juvenile mounting and rough play (Goy et al., 1988). These androgen treatments have not been reported to have any effect on male offspring, possibly because the serum androgen levels in male fetuses of treated mothers do not differ from the endogenous levels produced by fetal testes (Resko et al., 1987).

My laboratory has prenatally administered lower testosterone doses than used in previous monkey studies of prenatal hormonal influences on behavioral and anatomical sexual differentiation. These lower T doses model the effects of accidental androgen exposure. In addition there were treatment groups that received the antiandrogen flutamide to investigate the effect of blocking androgen receptors on sexual differentiation in male and female monkeys (Herman et al., 2000). Treatments were done on pregnant time-mated females (Zehr et al., 2000) living as members of 65–125-member social groups containing multiple adult males and females and their offspring. Table 2.2 presents the acronyms and treatments for each of the subject groups used in this study. Pregnant females received either testosterone enanthate (20 mg/week, intramuscular (IM) in oil vehicle) which should masculinize behavior or flutamide (30 mg/kg twice daily in dimethlyl sulfoxide (DMSO) vehicle) which could block any masculinization from the small amount of endogenous T that females are exposed to from their mothers, or vehicle (twice daily DMSO). The timing of treatments was varied such that about half of the subjects' mothers received 30- or 35-day-long hormonal treatments starting on either gestation day 35 or 40 through gestation day 70 (early treatments) or on gestation day 110 or 115 through gestation day 145 (late treatments). Thus the duration of treatment was the same for both early and late treatments. Only the time in gestation varied between the treatment groups. All treatments were administered within the pregnant female's social group and infants were delivered within the social group and mothers and offspring remained in the group for the duration of the longitudinal study.

Testosterone treatment produced maternal T levels ranging from 2.4 ng/ml to 21.7 ng/ml at the treatment nadir (Herman et al., 2000), which was substantially lower than reported in previous monkey studies (Resko et al., 1987). Females

Table 2.1 Summary of effects of prenatal hormonal manipulations in relation to dosage and gestational timing on anatomical and behavioral endpoints in male and female rhesus monkeys in studies where the subjects were not reared in socially restricted conditions

Prenatal treatment [references]	Sex	Genital anatomy	Rough play	Juvenile Mount
Early flutamide 35 or 40 days (Herman et al., 2000; Wallen, 2005)	♀	↑ Female-like	↔	↔
	♂	↓ Masculinized	Not different from control ♀ and ♂	Not different from control ♀ and ♂
Early testosterone 35 or 40 days (~3 mg/day) (Herman et al., 2000; Wallen, 2005)	♀	↔	Not different from control ♀ and ♂	↔
	♂	↔	Not different from control ♀ and ♂	↔
Late Flutamide 35 or 40 days (Herman et al., 2000; Wallen, 2005)	♀	↔	Not different from control ♀ and ♂	↔
	♂	↓ Penis length	↔	↑ mounts
Late testosterone 35 or 40 days (~3 mg/day) (Herman et al., 2000; Wallen, 2005)	♀	↔	↔	↔
	♂	↑ Penis length (not significant)	↑ Rough play	↔
Early testosterone 25 days (10 mg/day) (Goy et al., 1988; Goy, 1981)	♀	Masculinized	↔	↑ Mounts
Late testosterone 25 days (10 mg/day) (Goy et al., 1988; Goy, 1981)	♀	↔	↑ Rough play	↑ Mounts
> 50 days TP or DHTP (10 mg/day) (Goy, 1970; Goy, 1981; Goy & Phoenix, 1972)	♀	Masculinized	↑ Rough play	↑ Mounts
DESDP >100 days (Goy & Deputte, 1996)	♀	↔	↑ Rough play	> Control ♀ < Control ♂
	♂	↔	↔	↔
DESDP 25 days late gestation (Goy & Deputte, 1996)	♀	↔	↔	↔

Key: ♀ = female, ♂ = male, ↔ = no effect (Does not differ from same-sex control), ↑ = increased, ↓ = decreased
Not different from control ♀ and ♂ = subject values in between ♀ and ♂controls
DHTP 5α-dihydrotestosterone propionate, *TP* testosterone propionate, *DESDP* diethylstilbestrol dipropionate
(Wallen, 2005, copyright Elsevier, used with permission granted, Nov. 8, 2019)

Table 2.2 Abbreviations and factors for treatment groups in a study of the effects of prenatal hormone treatment and timing during pregnancy in group-living male and female rhesus monkeys

Group abbreviation	Timing of treatment	Type of treatment	Sex of subject
VCM	Early or late	Vehicle	**M**ale
VCF	Early or late	Vehicle	**F**emale
EAF	**E**arly GD[a] 35 or 40 until GD 75	**A**ndrogen (20 mg testosterone enanthate/week	**F**emale
LAF	**L**ate GD 110 or 115 until GD 150	**A**ndrogen (20 mg testosterone enanthate per week)	**F**emale
EFF	**E**arly GD1 35 or 40 until GD 75	**F**lutamide (30 mg/kg twice daily)	**F**emale
LFF	**L**ate GD 110 or 115 until GD 150	**F**lutamide (30 mg/kg twice daily)	**F**emale
EAM	**E**arly GD[a] 35 or 40 until GD 75	**A**ndrogen (20 mg testosterone enanthate/week	**M**ale
LAM	**L**ate GD 110 or 115 until GD 150	**A**ndrogen (20 mg testosterone enanthate per week)	**M**ale
EFM	**E**arly GD1 35 or 40 until GD 75	**F**lutamide (30 mg/kg twice daily)	**M**ale
LFM	**L**ate GD 110 or 115 until GD 150	**F**lutamide (30 mg/kg twice daily)	**M**ale

[a]*GD* gestation day

exposed to these levels of testosterone early in gestation (early androgen females or EAF) showed little evidence of genital masculinization, except for increased anogenital distance. In addition, their neonatal gonadotropin secretion was altered, suggesting that significant androgen had reached the fetus, but at levels below those necessary to masculinize genitalia (Herman et al., 2000). Females exposed to testosterone late in gestation (LAF) and females exposed to flutamide early (EFF) or late (LFF) in gestation showed no clear effects of treatment on genital anatomy or neonatal neuroendocrine function (Herman et al., 2000). Males exposed to flutamide early in gestation (EFM) had significantly less masculinized penises and, in one case, had a penis with a urethral meatus separate from the penile shaft as is typical of females. Thus, EFM penises were both smaller and less typically masculine than those of control males. Males exposed to flutamide late in gestation (LFM) had male-typical genitals, but their penises were significantly smaller than those of control males. Androgen treatment either early (EAM) or late (LAM) in gestation had no measurable effect on male genital anatomy, likely reflecting that the T dose was very small and didn't likely add to the endogenous levels from male's testes. The finding that flutamide treatment reduced penis masculinization either extensively (EFM) or in terms of penile size demonstrates that the penis remains sensitive to androgens after the prenatal period when the genital tubercle differentiates into the penis.

The range of prenatal treatments and the differing social conditions under which rhesus monkeys have been studied allow some generalizations about the role that prenatal androgens play in sexual differentiation of behavior. The sections that

follow are organized around specific behavioral endpoints. The specific nature of the behavioral sex difference is first described and then the effects of alterations in the prenatal hormonal environment are discussed. Although much work remains to be done to fully articulate the role that prenatal androgens play in behavioral differentiation, it is apparent that their effects are pronounced and that androgens are critical for masculinization of behavior, whether the endpoint is one requiring concurrent hormonal activation, like adult copulatory behavior, or a pattern not needing hormonal activation, like juvenile rough play.

2.5.1 Sex Differences in Juvenile Social Behavior

The finding of Phoenix et al. (1959) that prenatal hormones altered adult responsiveness to gonadal hormonal activation of adult sexual behavior led some to suggest that steroid hormones primarily organized sensitivity to hormonal activation (Whalen, 1968). While this applies to adult sexual behavior requiring hormonal activation in adulthood, the existence of sexually differentiated behavior that does not require hormonal activation for its expression provides the opportunity to distinguish the organization of behavioral patterns from the organization of sensitivity to hormonal activation. The demonstration by Harlow (1962) of sexually differentiated infant and juvenile behavior in monkeys provided an excellent behavioral system for investigating the effects of prenatal hormones on the organization of behavior. While several of the sex differences described by Harlow (1962), "rigidity," "threatening," and "withdrawal," are only seen in socially impoverished environments (Wallen, 1996), two patterns in particular, rough play and juvenile foot-clasp mounting as shown in Fig. 2.1, are important behavioral endpoints for demonstrating the effects of prenatal hormone manipulations.

Rough play (also called rough and tumble play, Fig. 2.1) is a high energy expenditure whole body play with grasping and tumbling that is exhibited more frequently by males than females in peer groups (Harlow & Harlow, 1962; Harlow, 1962; Goy, 1970), mother-peer groups (Wallen et al., 1977; Goy & Wallen, 1979; Wallen et al., 1981), and in large social groups (Lovejoy & Wallen, 1988; Wallen et al., 1995).

Juvenile rhesus monkey males display a variety of mounting postures, but of primary importance is the double foot-clasp mount (Fig. 2.1b) typical of the mating posture of adult male macaques (Altmann, 1962). This mount is displayed more by males than females (Lovejoy & Wallen, 1988; Wallen et al., 1995; Harlow, 1962; Goy, 1970; Harlow, 1965), but is only displayed with any appreciable frequency when males are reared with substantial opportunities for continuous social interaction with peers (Wallen et al., 1977; Wallen, 1996; Goy & Wallen, 1979; Wallen et al., 1981). In more socially limited contexts, foot-clasp mounting is almost never displayed (Harlow & Lauersdorf, 1974; Wallen et al., 1981; Harlow, 1965). Instead, in these socially restricted environments males will display mounts not oriented to the partner's pelvic region, will not display the double foot-clasp, and will often

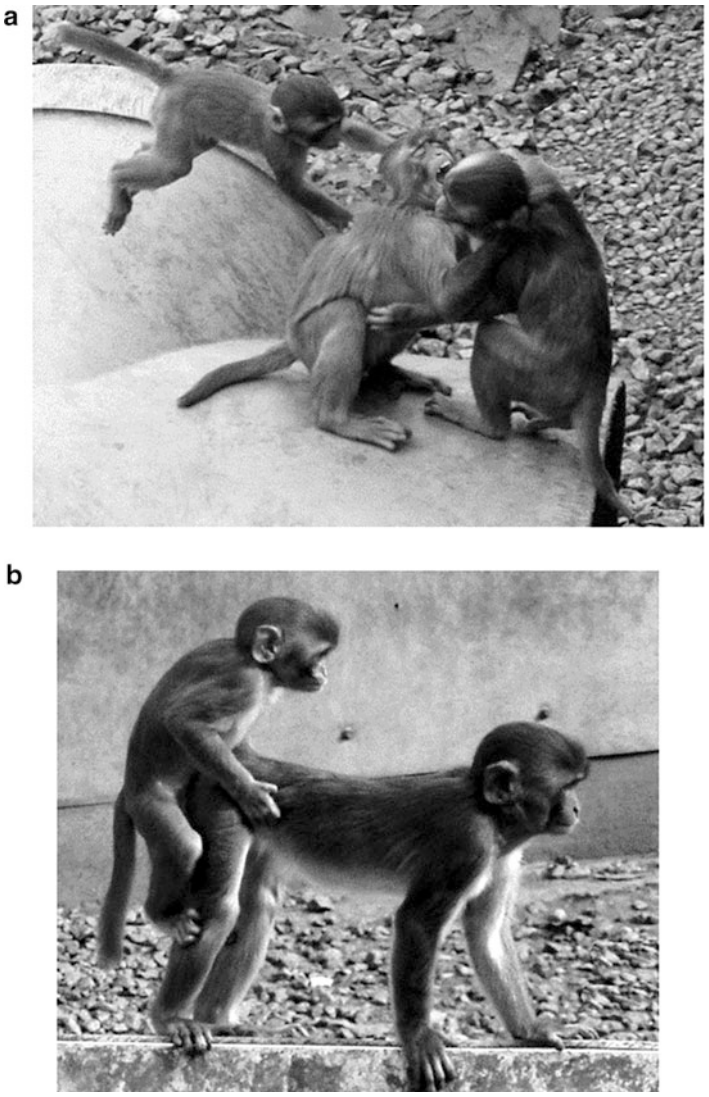

Fig. 2.1 (**a**) Rough play in yearling rhesus monkeys. The two male rhesus monkeys at the right engage in wrestling play, characterized by grappling and whole body involvement. Play may involve more than two animals as the infant on the left is about to demonstrate. (**b**) Foot-clasp mounting by an infant male to a yearling rhesus monkey. This mount is a highly cooperative behavior that is of the same form as that used by adult males during copulation. (Wallen, 2005, copyright Elsevier, used with permission granted by Elsevier, Nov. 8, 2019)

show mounts oriented to the partner's head or side (Wallen et al., 1977; Goy & Wallen, 1979). Unlike rough play, in which high frequencies occur under rearing conditions not conducive to developing adult social competence, the regular juvenile

display of foot-clasp mounting indicates juvenile socialization that predicts adult competency (Goy & Wallen, 1979; Wallen, 1996).

2.5.1.1 Studies of Effects of Exogenous Prenatal Steroid Administration on Rough Play and Mounting in Genetic Females

Early studies of the role of hormones on the development and expression of rough play and foot-clasp mounting demonstrated that their juvenile expression was not dependent upon the presence of male gonadal function as neonatally castrated males displayed these behaviors at levels indistinguishable from gonadally intact males (Goy, 1970). In contrast to the lack of effect of postnatal androgenic influences, these behaviors were significantly increased in genetic females whose mothers had been treated with either testosterone or 5α-dihydrotestosterone, a nonaromatizable androgen, during much of gestation (Goy, 1970; Goy, 1981; Goy & McEwen, 1980; Goy & Resko, 1972). Japanese macaque females whose mothers received testosterone from approximately gestation day 40 to 100, of the 160-day gestation, showed increased mounting, but not rough play compared to control females (Eaton et al., 1990). The androgen levels in this study were lower than those employed by Goy and colleagues in the rhesus monkey, as the female Japanese macaque offspring exposed to androgen in utero did not have extensive genital masculinization. These results suggest that lower levels of prenatal androgen are required to masculinize mounting than are required to masculinize rough play. Further evidence that play and mounting have different sensitivities to androgens came from a study in which androgen exposure was limited to a 25-day period during gestation, but varied the gestational timing of the 25-day treatment (Goy et al., 1988; See Table 2.1 for a summary of findings).

Exposing genetic females to 10 mg of testosterone propionate (TP) injected daily to their mothers on gestation days 40–64 extensively masculinized their genitalia, producing a fully formed penis, scrotum, and no vaginal opening. Behaviorally, these genitally masculinized females mounted at higher frequencies than did control females and did not differ from control males. However, these early androgenized females, like Eaton's Japanese macaques exposed to lower levels of T, did not show increased frequencies of rough play (Goy et al., 1988). By contrast, the same dose of T administered from gestation days 115–139, produced no detectable masculinization of the female's genital anatomy, but significantly elevated both mounting and rough play compared to control females, and the elevated rough play was over the levels displayed by early androgen-treated females (Goy et al., 1988). Thus, the timing of androgen exposure separated its effects on genital anatomy from its effects on behavior. This study also suggested that late gestation is a period of particular sensitivity of the developing nervous system to prenatal androgens. This might be expected since the time course of genital differentiation differs markedly from that of neural differentiation. While genital differentiation is completed by approximately gestation day 75, cortical neurons have not completely proliferated in some areas of the macaque brain until after gestation day 100 (Rakic, 1988). In addition,

synaptogenesis of these neurons, which begins after prenatal gonadal differentiation, continues through the first two postnatal months (Bourgeois et al., 1994; Granger et al., 1995). Evidence that synaptogenesis can be influenced by androgens (Matsumoto, 1991) provides support for the latter part of gestation being a period of particular sensitivity to androgens for behavioral differentiation. Studies in humans show a pattern of prenatal and postnatal synaptogenesis similar to that seen in rhesus monkeys (Huttenlocher & Dabholkar, 1997). The timing in humans and monkeys is also similar, when considered in relation to the longer human gestation.

Both aromatizable and nonaromatizable androgens masculinize juvenile behavior. Because estrogen levels in females are typically higher than in males, estrogens have been thought to produce feminization. There is, however, little evidence in support of this view (Fitch & Denenberg, 1998). There is some evidence suggesting that estrogens can masculinize rhesus monkey juvenile behavior (Table 2.1). Goy and Deputte (1996) treated pregnant females for more than 100 days of gestation with 100ug per day of the synthetic estrogen, diethylstilbestrol dipropionate (DESDP), a dose 33 times that shown to masculinize and defeminize aspects of the behavior of genetic female guinea pigs (Hines et al., 1987). Females experiencing long DESDP treatment had female-typical genitalia, but displayed increased juvenile mounting and rough and tumble play. Another group of females received shorter DESDP treatments timed similarly to the late gestation androgen treatments described above. These short-term treated DESDP females showed no evidence of behavioral masculinization, in contrast to the significant masculinization produced by short TP treatments late in gestation. DESDP females were only studied for their first year of life while still in the presence of their mothers when the full expression of juvenile sex differences has yet to be realized (Goy & Wallen, 1979). Thus, it is hard from these data to determine whether the masculinization produced by long-term treatments with large amounts of DESDP reflect an involvement of estrogens in masculinization, or a pharmacological effect of this synthetic compound. Clearly, the DESDP treatment had the capacity to influence sexual differentiation, but the short-term nature of the study period in this single report prevents reaching any definitive conclusions about the role that estrogens play in the sexual differentiation of juvenile behavior in rhesus monkeys.

DESDP is probably the only estrogenic hormone that can be used to investigate the role of estrogens in sexual differentiation, because, unlike estradiol, DESDP doesn't induce abortion when administered to pregnant females. That estrogens typically induce abortion in primates, including humans, accounts for why there has been so little work with prenatal estrogens in primates. Because in altricial rodents sexual differentiation occurs mostly after birth means that abortion is not an issue in rodent studies. Whether or not estrogens or estrogenic metabolites play any role in the normal course of juvenile behavioral differentiation in primates as it does in some rodent species (Wallen & Baum, 2002) remains an open question.

2.5.1.2 Effects of Altering Endogenous or Exogenous Androgens on Rough Play and Mounting in Males and Females

The hypothesis that late gestation is a period of increased sensitivity to the organizing actions of androgens on sexually differentiated behavior was investigated in an omnibus study that use lower doses of exogenous testosterone than employed in the studies of the previous section, and by interfering with the actions of endogenous androgens using the antiandrogen flutamide (Herman et al., 2000). This is the first study to attempt to alter endogenous androgen actions in monkeys. This manipulation potentially allows studies of hormonal influences on sexual differentiation to reveal normative mechanisms of masculinization.

Subjects were the offspring of mothers receiving vehicle, flutamide, or testosterone enanthate early or late in gestation as shown in Table 2.1. The treatments and observation procedures have been previously described (Herman et al., 2000; Herman et al., 2003; Tomaszycki et al., 2001). Early flutamide treatment of males (EFM) prevented full masculinization of the male's penis, whereas late flutamide treatment resulted in a fully formed, but significantly smaller penis. Early flutamide treatment in females (EFF) modified their genitals in a more female-typical direction suggesting that females are exposed to some level of endogenous androgens. None of the TE treatments significantly affected genital anatomy in either male or female offspring, although males exposed to androgen late in gestation (LAM) had the longest penises of any male group (Herman et al., 2000). The results from the study described above have been previously reported (Wallen, 2005, 2009, 2017) and are briefly recapitulated here. The study results are presented for each of the first 2 years of life. The years are presented separately because rhesus monkeys interact extensively with their mothers during the first year of life, but spend substantial time away from their mother during the second year of life and beyond. Thus treating the 2 years separately reflects developmental changes that occur in the 2 years.

First year of life: Behavioral data were collected as previously described for the first 6 months of the first year of life (Herman et al., 2000; Herman et al., 2003; Tomaszycki et al., 2001).

During the first 6 months of life infants spend much of their time with their mothers. When the data on rough play are characterized by whether they are within 1 m or less from their mother juveniles display low incidence of rough play and there is no statistically significant sex difference. By contrast, when play is measured when infants are more than 1 m from their mothers, a significant sex difference in rough play is seen. This may reflect that males spend more time away from their mothers than do females.

Behavioral data were analyzed using a one-way analysis of variance (anova) with treatment group (Table 2.2) as the factor followed by multiple Bonferroni post hoc comparisons. Rough play differed significantly across the 10 treatment groups with all male groups displaying significantly higher rates of rough play than did any of the female groups (see Table 2.2 for group descriptions). Although there was an overall main effect of prenatal treatment group ($F(9, 52) = 6.36$, $p < 0.001$) on rough play

rates, female rough play was not increased by treatment with prenatal testosterone (EAF and LAF groups) compared to play rates of control females. The only suggestion of a prenatal hormonal effect in females was that late-treated females (LAF and LFF subjects, Table 2.2) played at frequencies not significantly different from either control males or control females. In males, both early and late androgen-treated males displayed higher rates of rough play than did any female group with the exception of the LFF subjects who did not differ significantly from control males. Contrary to predictions, administration of flutamide to males late in gestation (LFM, Table 2.2) did not significantly decrease rates of rough play. Although early flutamide treatment of males (EFM, Table 2.2) produced the lowest rates of male rough play, EFM subjects did not differ significantly from any other treatment group. Thus their play was not significantly more masculinized than that of females nor significantly less masculinized than that of the other males, an unexpected finding.

Foot-clasp mounting is very infrequent during the first 6 months of life, so the measure presented here includes all properly oriented mounts (penis in correct juxtaposition to the rear of the animal being mounted), not just mounts with foot-clasps. There was an overall treatment effect $F(9, 52) = 2.92$, $p = 0.007$ reflecting, surprisingly, that late flutamide-treated males (LFM, Table 2.2) mounted more than did either control males or any of the female groups. Thus contrary to our hypothesis that flutamide late in gestation would block juvenile behavioral masculinization, it paradoxically seems to have hypermasculinized these males, an effect not evident in EFM subjects who, as with rough play, did not differ significantly from any other group. There was no evidence that prenatal androgen exposure affected female mounting rates.

These findings of sex differences in the first year of life are consistent with findings in studies under more limited social conditions (Wallen, 1996). The effects of testosterone treatments differed from previous studies principally because we used T doses that did not affect genital masculinization. The flutamide treatment was unique and reduced genital masculinization in males, but paradoxically produced increased masculinization of behavior when given late in gestation. Infant monkeys spend substantial time with their mother during the first year of life resulting in low frequencies of both mounting and rough play, possibly making it less likely to identify hormonal influences on these behaviors. This is not the case for the second year of life where juvenile monkeys spend much of their day completely independent of their mother.

Second year of life: Behavioral data were collected as previously described (Wallen, 2005) for 13–15 weeks starting on the subject's first calendar birthday, which is thought to developmentally represent 4 years in human time. Data were analyzed as previously described (Herman et al., 2000; Herman et al., 2003; Tomaszycki et al., 2001). Data were collapsed across all weeks of observation to provide a total occurrence of the specific behavior.

Rough play varied with prenatal treatment ($F(9, 48) = 6.91$, $p < 0.001$) with control males playing more than did control females (Fig. 2.2a). Among the female treatment groups, only EAF subjects displayed significantly less rough play than did control males with the other female treatment groups differing significantly from

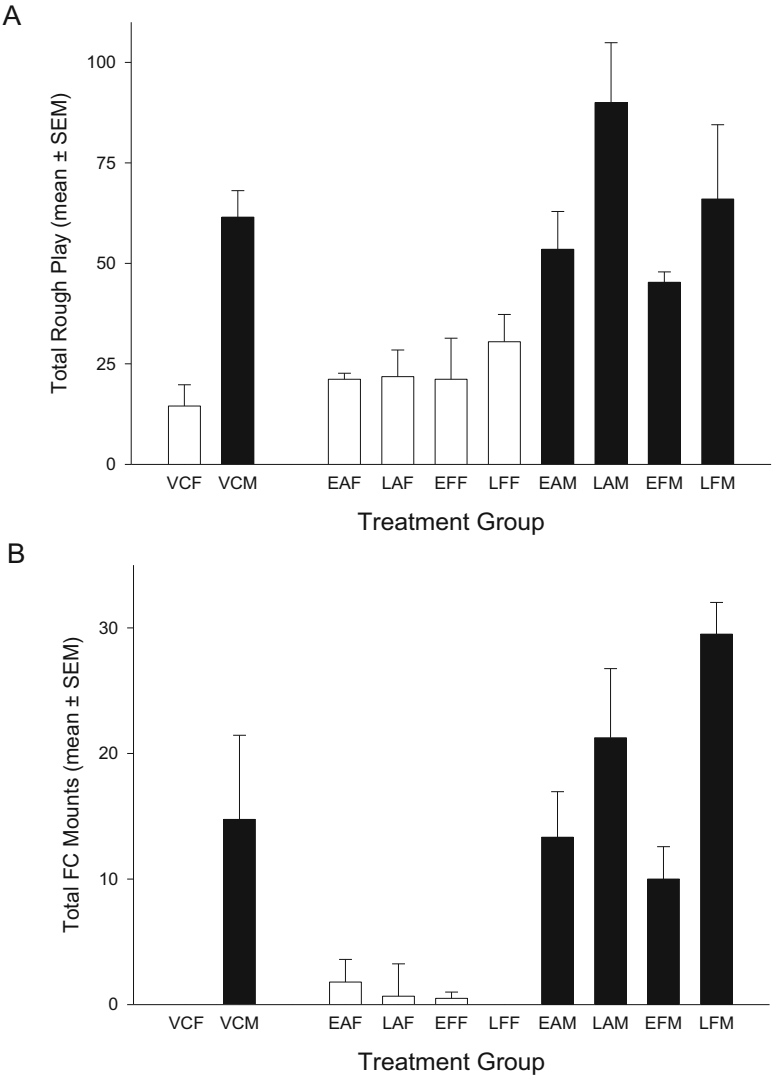

Fig. 2.2 Rough play (**a**) and mounting (**b**) displayed by control and prenatally treated rhesus monkeys during the second year of life. Abbreviations: *VCF* vehicle-treated control female, *VCM* vehicle-treated control male, *EAF* early-treated androgen female, *LAF* late-treated androgen female, *EFF* early-treated flutamide female, *LFF* late-treated flutamide female, *EAM* early-treated androgen male, *LAM* late-treated androgen male, *EFM* early-treated flutamide male, *LFM* late-treated flutamide male (see text for details of treatments). (Wallen, 2005, copyright Elsevier, used with permission granted by Elsevier, Nov. 8, 2019)

either control males or females (Fig. 2.2a). Thus prenatal testosterone treatments to females, except EAF, increased rough play sufficiently such that females were neither completely masculine nor feminine in their pattern of play. Surprisingly,

late flutamide treatment had the most pronounced masculinizing effect on female rough play, although this was not significantly different from any other female or male group. In addition to control males, males receiving prenatal hormonal treatments late in gestation, regardless of whether the treatment was T or flutamide, were the only male treatment groups to differ from control females. In addition, LAM subjects differed significantly from all prenatally treated female groups (Fig. 2.2a). LFM subjects differed significantly from EAF subjects in addition to control females, making them the most fully behaviorally masculinized of the male treatment groups after LAM subjects. In contrast to the LAM and LFM subjects, early male treatment groups (EAM and EFM, Table 2.2) did not differ significantly from any other group in their rough play. Thus, like the late flutamide females, these groups of males were neither fully masculine nor feminine in their play patterns, suggesting that these early prenatal treatments, both androgen and flutamide, had partially blocked full masculinization.

Foot-clasp mounting also varied with prenatal treatment. Control males displayed significantly more mounts than did control females (Fig. 2.2b) and more than any female treatment groups (Fig. 2.2b). None of the female treatment groups differed significantly from the control females (Fig. 2.2b); thus unlike the case of rough play, there was no evidence that prenatal androgen or flutamide treatment had any impact on the occurrence of juvenile mounting by females. By contrast, manipulating androgens in males significantly affected their mounting behavior. Late treatments, either with T or flutamide, produced elevated levels of mounting (Fig. 2.2b). Males receiving flutamide late in gestation (LFM) showed the highest level of mounting of any group in the study and differed significantly from all groups except the late androgen-treated males (LAM, (Fig. 2.2b). In contrast, flutamide treatment given early in gestation (EFM) produced males whose mounting did not differ significantly from any group, male or female. Late androgen-treated males (LAM) mounted at higher frequencies than any of the female groups, but not more frequently than any other male treatment group. Males receiving androgens early in gestation (EAM) were similar to late-treated males (LAM), differing only in that they did not mount more frequently than did EAF and mounted less frequently than did late-treated flutamide males (LFM, Fig. 2.2b).

These results are surprising and support several conclusions. First, juvenile male mounting is particularly sensitive to hormonal manipulations during late gestation. In fact, the only effects of either androgen or antiandrogen treatment were in late gestation treatments, suggesting that this time in gestation is when the developing nervous system underlying behavior is organized by steroid exposure. Second, paradoxically, both flutamide and exogenous T had similar effects when administered late in gestation, with both significantly augmenting mounting. It is unlikely that this reflects that masculinization does not involve the activation of androgen receptors (AR), but instead that flutamide has complex effects in males with an intact hypothalamic, pituitary, testicular (HPT) axis that may, paradoxically, increase testosterone levels by blocking testicular negative feedback. Last, differentiation of mounting is somewhat sensitive to hormonal variation early in gestation as well, with early flutamide treatment producing males (EFM) with the poorest mounting

performance. These males also had the least masculinized genitals raising the possibility that the lower mounting displayed by EFM subjects reflected reduced sensory feedback from their less completely masculinized penile structure.

The effects that we produced using prenatal flutamide treatments are paradoxical and contradictory if one assumes that flutamide works consistently as an antiandrogen and in the same manner on both neural and peripheral organ systems. There are several possible explanations for these findings. One possibility is that flutamide simply doesn't enter the brain in sufficient amounts to occupy a significant proportion of brain androgen receptors. No in vivo studies have been done that administer flutamide peripherally (e.g., not directly administered into the brain) and measure brain levels of flutamide compared to peripheral levels. Since flutamide is a relatively weak AR ligand (Singh et al., 2000), any decreased neural flutamide concentration could markedly reduce its antiandrogenic behavioral effects. Probably more critically, flutamide treatment can suppress LH negative feedback resulting in increased LH secretion (Grattan et al., 1996; Sodersten et al., 1975; Veldhuis et al., 1992). This increase in LH potentially increases endogenous androgens making a neural flutamide antiandrogen blockade even less effective.

In a study of intact male rats (Sodersten et al., 1975), peripherally administered flutamide produced no measurable reduction of male sexual behavior. Flutamide treatment of intact males markedly increased LH and T, reflecting suppressed negative feedback. These same males, however, had inhibited prostatic growth demonstrating a peripheral effect of flutamide. Castrated males, who produce no endogenous T, treated with flutamide and T showed reduced ejaculations, but no change in intromissions or mounts, suggesting that flutamide was not having a clear effect on the brain. By contrast, prostate growth in these T-treated castrated rats was completely inhibited by concurrent flutamide treatment (Sodersten et al., 1975). Taken together, these results suggest that flutamide can block androgenic effects on androgen-sensitive peripheral structures without blocking centrally mediated androgen-sensitive behaviors.

One study of pregnant rats administered flutamide found it blocked the masculinization of the corpus callosum in male offspring (Fitch et al., 1991). There is, however, scant additional evidence that peripherally administered flutamide has significant effects on brain structure and function. Thus one possible explanation for the behavioral masculinization effects that we found with peripherally administered flutamide is that flutamide effectively blocks testicular negative feedback, resulting in either increased steroid secretion in pregnant females, such as the increased T secretion we found in our flutamide-treated mothers (Herman et al., 2000), or produces an increased secretion of testicular T in fetal male offspring of flutamide-treated mothers. In this regard, it may be significant that neonatally, LFM subjects had significantly higher T levels than any other group during the first 2 weeks postnatally (Herman et al., 2000). Whether this reflects alteration in testicular sensitivity to gonadotropins (LFM subjects did not have elevated LH when their neonatal T was elevated), or some remaining effect of the late flutamide treatment on hypothalamic-pituitary-gonadal function (HPG), remains to be seen.

Flutamide did cross the placenta as shown by its blocking full masculinization of male genitalia in the EFM group. However, behavioral masculinization was not blocked, and in fact was enhanced in some cases, a finding incompatible with significant levels of flutamide entering the developing brain.

An alternative possibility is that flutamide interferes with testicular negative feedback elevating androgens, but that estrogenic metabolites from aromatization of these elevated androgens produce increased behavioral masculinization. Given that prenatal DHT (which cannot be aromatized to an estrogen) both masculinizes and defeminizes the sexual behavior of adult female rhesus monkey (Thornton et al., 2009) and given the moderate effects of the synthetic estrogen DESDP on juvenile behavioral masculinization, aromatization of T seems an unlikely explanation for our findings. It cannot, however, be ruled out at this time. It is apparent from our results that the administration of flutamide to animals with an intact HPG axis does not produce results consistent with flutamide acting on the brain and having a pure antiandrogenic mode of action. In some ways, this should not be surprising given that one of the earliest studies of flutamide in intact male rats reported no antiandrogenic effect on male sexual behavior (Sodersten et al., 1975).

2.5.1.3 Sex Differences in Juvenile Interest in Infants

As seen in adults, juvenile and prepubertal females exhibit much greater interest in infants than do juvenile and prepubertal males in several primate species (Herman et al., 2003), including humans (Feldman et al., 1977; Maestripieri & Pelka, 2002). Most of the studies of prenatal hormone manipulations were done in social contexts precluding measurement of interest in infants and thus we know nothing about the effect that long T or DHT treatments may have had on this endpoint. In contrast, subjects in Herman et al. (2003) were reared in complex social groups, where access to infants was a typical aspect of the environment. Subjects were those previously described for the studies of juvenile mounting and rough play described above. A variety of measures of infant interest were collected during approximately 10 hrs/yr. of 15 min focal observations of social behavior for each of the first 3 years of life on each subject: Behavioral measures included touching, holding, playing with infants, and kidnapping infants (Herman et al., 2003). Sex differences in interactions with infants are striking, with effect sizes (Cohen's d) ranging from 1.3 (frequency of kidnapping in yearling subjects) to 5.1 (frequency of touching infants in yearling subjects). These are among the largest behavioral sex differences reported in rhesus monkeys, or any other species for that matter. Together, 14 measures differed significantly between males and females, but few of these measures were affected by prenatal treatment to females and none affected males.

Surprisingly, females receiving flutamide late in gestation (LFF) showed masculine patterns of infant interest on five of the 14 measures, very much like what we found for mounting behavior in LFM. To maximize the power of our multiple measures we calculated an index of infant interest that used the deviations from the control males and females across all measure differing significantly between

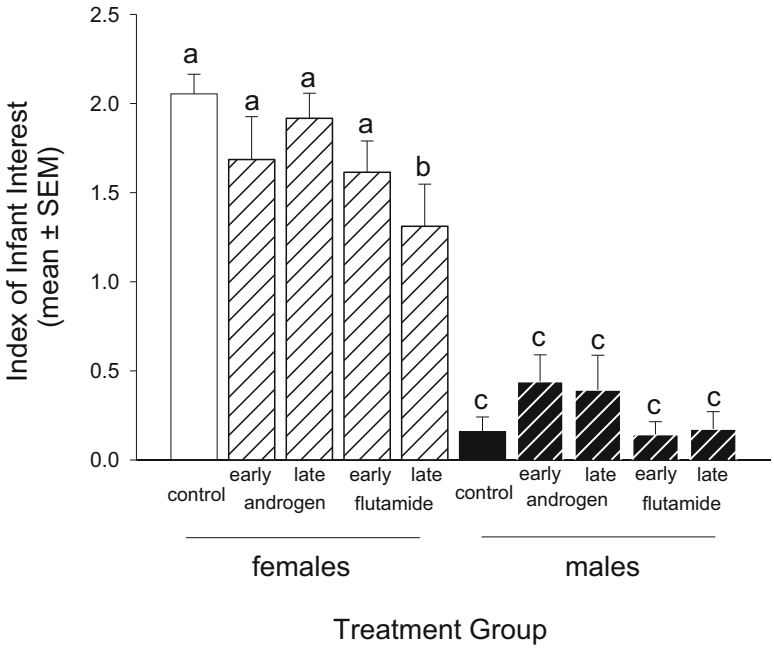

Fig. 2.3 Index of interest in infants for control male and female rhesus monkeys and males and females receiving testosterone or flutamide treatments early and late in gestation. Bars with differing superscripts differ significantly. (Herman et al., 2003, copyright Elsevier, used with permission granted by Elsevier, Nov. 11, 2019)

males and females (Herman et al., 2003). Figure 2.3 illustrates the effect of prenatal treatment on this measure of infant interest. Males differed significantly from all female groups, but the LFF group differed significantly from all other female and male groups, suggesting that they showed less interest in infants than did control or EFF females, but more interest in infants than did males (Herman et al., 2003). This supports juvenile interest in infants reflecting prenatal hormonal action late in gestation, but suggests that our treatments were near the threshold for effectiveness in altering interest in infants. It is important to point out that the effect of late gestation flutamide was consistent with androgen defeminization of interest in infants. This may reflect that flutamide treatment increased maternal T and thus may have elevated T in the fetus. That the action of this maternal T that got to the fetus was apparently not blocked in the fetus by the flutamide treatment is consistent with insufficient flutamide entering the brain to block the effects of elevated maternal T.

Juvenile interest in infants is of particular interest because of the magnitude of the sex difference and that it occurs at a time when the gonads are quiescent, arguing that it is the expression of an underlying behavioral predisposition not requiring hormonal activation. Particularly intriguing is that interest in infants in post-pubertal reproductive females, unlike in juvenile females, appears to be activated by ovarian

hormones under our social conditions (Maestripieri & Zehr, 1998; Maestripieri & Wallen, 1995). Those hormones do not modulate infant interest before puberty, but do after puberty as is also seen in male mounting. Juvenile mounting, like juvenile interest in infants, requires no hormonal activation, whereas adult copulatory mounting does (Wallen, 2001). The mechanism by which a behavior occurs prior to puberty without hormonal activation and then comes under hormonal activational control post-pubertally has not been investigated. It is likely, however, that understanding the mechanism of this transition will be important to developing a general understanding of hormonal modulation of behavior.

2.5.1.4 Juvenile Sex Differences in Preferences for Human Toys

A striking sex difference among children is preference for stereotypical masculine and feminine toys (Berenbaum & Hines, 1992). This sex difference has often been presented as evidence of the social construction of human sex differences and has been thought to reflect parental endorsement of sex-typed toys and encouragement of boys and girls to play with different toys (Martin & Little, 1990). An alternative view is that toy preferences reflect activity preferences and that the sex difference in toy preferences reflects that boys and girls have different activity preferences. If this is the case and if monkeys share this same sex difference in activity preferences, they possibly could show similar preferences for human sex-typed toys as do boys and girls. This notion was first explored by Melissa Hines and Gerianne Alexander (Alexander & Hines, 2002) who measured play times for male and female vervet monkeys with sex-typed human toys. Monkeys were given, in randomized serial presentation, either a stereotypical male toy (truck or ball), a stereotypical female toy (doll or cooking pan), or a gender-neutral toy (picture book or furry dog), and how long they played with the toy was recorded. The gender-neutral toys showed no sex differences, but male vervets played more with stereotypical male toys than did female vervets, though males did not differ in play with the male and female toys. Female vervets played more with the stereotypically female toys than did male vervets and females also played more with the female toys than they did with male toys. Because these play times were collected with only a single toy in the cage, this study didn't directly test preference although it likely reflects interest in the toy type as the monkey could choose to not play with the toy if uninterested. These results provide some support for the notion that vervet monkeys express a sexually differentiated interest in human toys that is similar to those expressed by boys and girls. The differences between these results in vervets and those in children could reflect that toys in the vervet study were presented serially whereas in human studies children have access to multiple toys and thus can clearly demonstrate a preference for a sex-typed toy.

We followed Alexander and Hines' study with a toy-preference study in group-living rhesus monkeys (Hassett et al., 2008) in which members of a social group of monkeys had access to both a wheeled toy (cars, trucks, a wagon, shopping cart) and a plush toy (dolls, stuffed animals) and were free to interact with whichever toy they

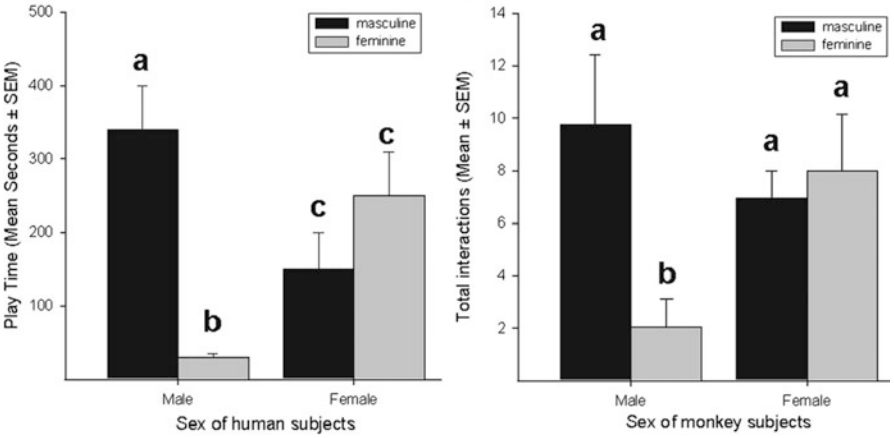

Fig. 2.4 Sex differences in interaction with sex-typed toys in children (left side, adapted from Berenbaum & Hines, 1992) and juvenile rhesus monkeys (right side, adapted from Hassett et al., 2008). Children's toys consisted of an array of stereotypical boy's and girl's toys. The monkey's toys were either wheeled toys (masculine) or plush toys (feminine). Monkeys had access to either toy in their social group. Superscripts that differ within a figure indicate significant differences. Bars with the same superscript do not differ significantly. Both boys and male monkeys show a strong preference for masculine toys and interact little with feminine toys. By contrast, girls and female monkeys show a weak and nonsignificant preference for feminine toys. Girls show less interest in masculine toys and more interest in feminine toys than do boys. In monkeys, females show more interest in feminine toys than do male monkeys, but do not differ from male monkeys in interacting with masculine toys. (Hassett et al., 2008, copyright Elsevier, used with permission granted by Elsevier, Nov. 8, 2019)

preferred. These two categories of toys were selected because they loosely divided the toys in masculine and feminine toys, but also because they facilitated very different interactions with the toys. There was clear evidence of sex differences in toy preference with male monkeys strongly preferring the wheeled toys and females showing a moderate preference for the plush toys (Fig. 2.4). These sex differences in rhesus monkey toy interactions largely duplicated the sex differences seen in children in a study by Berenbaum and Hines (1992). The striking differences in both species are that males show a very pronounced preference for the male-typical toys, but females, while preferring, to some extent, female-typical toys don't show a statistically significant preference for either toy type. In other words, both boys (Berenbaum & Hines, 1992; Meyer-Bahlburg et al., 2004) and juvenile male monkeys (Hassett et al., 2008) show very stereotyped toy preferences, whereas girls and juvenile monkeys are much less pronounced in their toy preferences. The similarity in response by children and juvenile monkeys is striking because the toys in the two studies were different. Because monkey toy preferences could not have been socialized (the toys were novel to the monkeys), these results could support the notion that the sex difference in toy preferences reflects that boy's and girl's toys promote different activities (wheeled toys promote manipulation and large motor movements, whereas plush toys promote protosocial interaction) that appeal differently to males

or females instead of reflecting a preference for a "boy's" or "girl's" toys per se, distinctions not available to monkeys. We know that prenatal androgens promote different activities in male and female juvenile monkeys, but whether such prenatal androgen exposure predisposes males to prefer the different activities that toys promote remains to be investigated.

2.6 Conclusion

Prenatal exposure to supraphysiological levels of exogenous androgen (either testosterone or 5α-dihydrotestosterone) during the second or last third of gestation masculinizes juvenile mounting and rough play behavior of genetic rhesus monkey females. Importantly, only treatments during the second third (second trimester) of gestation masculinize both female genitalia and mounting behavior, whereas treatments in the last third of gestation (third trimester) don't masculinize genitalia, but have the biggest effect on behavior masculinizing both mounting and rough play. Thus, genital and behavioral masculinization are separable processes and masculinization of behavior does not require genital masculinization. Similarly, blocking endogenous androgen in genetic males during the second third of gestation significantly reduced genital masculinization, but did not prevent masculinization of behavior, again demonstrating the independence of genital and behavioral masculinization. Whether flutamide blockade failed to prevent masculinization of behavior because it did not reach the brain in sufficient quantities or because estrogenic metabolites, which would not have been blocked by flutamide, are important for male masculinization remains to be resolved.

Across studies, it does appear, however, that estrogens are not critical to male sexual differentiation, although there are still too many gaps in the data to be completely confident of this conclusion. However, it is apparent that the nonaromatizable androgen, DHT, both masculinized and defeminized the behavior of genetic females when administered prenatally. Thus, it seems likely that sexual differentiation in the precocial rhesus monkey is more similar to the precocial guinea pig than it is to the other altricial laboratory animals (Wallen & Baum, 2002). In both rhesus monkeys and guinea pigs, prenatal DHT masculinizes female sexual behavior, whereas in altricial species like the rat, DHT does not masculinize female behavior unless estrogen is also given (Wallen & Baum, 2002). In contrast to the guinea pig, where prenatal DHT does not defeminize genetic females (Goldfoot & van der Werff ten Bosch, 1975), prenatal DHT treatment defeminized female proceptive behavior in rhesus monkeys (Pomerantz et al., 1985). Whether this reflects a true developmental species difference or a difference between the hormonal influences on receptivity in the guinea pig and proceptivity used in rhesus monkeys remains to be resolved. Taken together, it seems unlikely that estrogenic metabolites of testosterone are the active agents stimulating behavioral sexual differentiation in rhesus monkeys.

The hormonal manipulations that are possible in rhesus monkeys, but not in humans, can guide us to understanding the likelihood that similar processes operate in humans. Specifically, monkey studies can control the amount and timing of hormonal manipulations in ways impossible on humans. What findings there are in humans come primarily from natural variations in hormones and from two clinical conditions: Congenital Adrenal Hyperplasia (CAH), in which female fetuses are exposed to elevated prenatal androgens (though at levels lower than those experienced by genetic males) and exhibit male-typical behavior in certain aspects of development, and Complete Androgen Insensitivity (CAIS), in which genetic males lack androgen receptors and thus their endogenous androgens cannot influence their sexual development resulting in nearly complete feminized genitals and female-typical behavior. Thus, it does seem likely that humans would show similar responses to exogenous androgens during gestation as those found in monkeys.

It is apparent from these studies that the latter part of gestation is an important period for prenatal hormones to affect brain organization in rhesus monkeys. Consistently across studies using high levels of testosterone, or our studies using lower dosages and antiandrogen treatment, behavioral effects late in gestation were more pronounced than those seen in early gestation. Thus, it seems that this period of significant synaptogenesis (Bourgeois et al., 1994; Granger et al., 1995) is also an important period for behavioral differentiation.

The effects of prenatal hormones on behavioral differentiation are profound and significantly determine developmental trajectories in both male and female rhesus monkeys. The consistent findings of effects on mounting and rough play across different social contexts suggest that these behaviors are particularly sensitive to prenatal hormonal influences. However, it is important to remember that social context also significantly affects sexually differentiated behavior. Other patterns of behavior, such as threatening behavior, are sexually differentiated in some social conditions but not others, and prenatal hormones do not consistently affect the development of this behavior (Wallen, 1996). Similarly, prenatal androgens appear to have little effect upon adult copulatory behavior of females reared under restrictive social conditions (Phoenix et al., 1959; Phoenix et al., 1983), but profoundly alter female copulatory behavior when reared under less restrictive conditions (Pomerantz et al., 1985; Pomerantz et al., 1986; Thornton & Goy, 1986). This effect of social context on steroid action likely reflects that socially restrictive conditions suppress the development and expression of sex-specific behavioral predispositions. This behavioral suppression is large enough that steroid modulation of these sexually dimorphic behaviors is difficult or impossible to see. The suppressive effect of restrictive social environments likely reflects that social restriction alters social interactions whether or not they require hormones. Thus, the effect of prenatal hormonal manipulations reflects an interaction between the specific hormonal manipulation, its timing in gestation, and the social history of the animal. Ultimately, sexually differentiated behavior reflects both the hormonally organized predisposition to engage in a behavior and the social experience and current social context to convert that predisposition into behavioral expression.

Are these findings relevant to humans? While one cannot extrapolate directly from nonhuman primates to humans, these results raise issues relevant to humans. The most important of these is the finding that late gestation exposure to elevated prenatal androgen fully masculinizes aspects of female juvenile behavior without detectable genital masculinization. If similar processes pertain to humans, it is possible that there are conditions where genetic females are psychologically, but not genitally, masculinized. Similarly, in genetic males, interfering with androgen function late in gestation could reduce or block psychological masculinization without modifying male genitalia. Such late-gestation psychological effects could be one basis for human transgenderism, where there is psychological cross-gender identification and behavioral expression without genital modification.

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Spotlight Feature: The Implications of Social Environment for Sex Differences in Brain and Behavior

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Naked mole-rats (*Heterocephalus glaber)* do not fit the traditional framework of mammalian sexual development in that most individuals remain pre-pubertal for the duration of their [extraordinarily long] lives (>28 years) (Buffenstein, 2005; Jarvis, 1981). Native to East Africa, naked mole-rats are small eusocial rodents, living in underground colonies of up to 300 individuals (Jarvis, 1981). Eusociality refers to their rigid social hierarchy where breeding is restricted to one dominant female (the queen) and 1–3 males. All other colony members are socially subordinate and reproductively suppressed. These "subordinates" are remarkably sexually monomorphic for adults of a sexually reproducing species, failing to show many of the sex differences in behavior, gross morphology, endocrinology, and neural morphology (Holmes et al., 2009) that are highly conserved in mammals. For example, subordinates of both sexes participate equally in both prosocial and agonistic interactions (Lacey & Sherman, 1991; Mooney et al., 2015). Male and female subordinates are of similar body size and weight (Dengler-Crish & Catania, 2007; Peroulakis et al., 2002) and, unlike other rodents, there is no sex difference in anogenital distance, with possible male feminization of the genitalia such that the external penis resembles a clitoris in shape and size (Jarvis, 1981; Peroulakis et al., 2002; Seney et al., 2009) (Fig. 2.5). Furthermore, the perineal muscles used in copulation are sexually monomorphic in subordinate naked mole-rats despite showing dimorphism in most mammalian species (Peroulakis et al., 2002). Finally, no sex differences are seen in circulating gonadal steroid hormones in subordinates (Clarke & Faulkes, 1998; Faulkes, Abbott, & Jarvis, 1990; Swift-Gallant et al., 2015; Zhou et al., 2013), and we failed to find sex differences in regional brain volume, cell number, or cell size in reproductively relevant brain regions known to be sexually differentiated in other mammals (e.g., medial amygdala) (Holmes et al., 2007).

Crucially, subordinate naked mole-rats are neither asexual nor sterile. They can become reproductive if removed from the suppressive influence of the queen, showing the endocrine and behavioral transitions characterized as mammalian puberty. We often see these changes occurring in both males and females. For example, RFamide-related peptide-3 immunoreactivity (the mammalian ortholog of gonadotropin inhibitory hormone) is lower in breeders than subordinates, regardless of sex, in the dorsomedial and arcuate nuclei of the hypothalamus (Peragine et al., 2017). This protein is thought to be a main player contributing to reproductive suppression in subordinates. Similarly, breeders of both sexes have larger regional volumes of the medial amygdala, bed nucleus of the stria terminalis, and paraventricular nucleus of the hypothalamus when compared to subordinates.

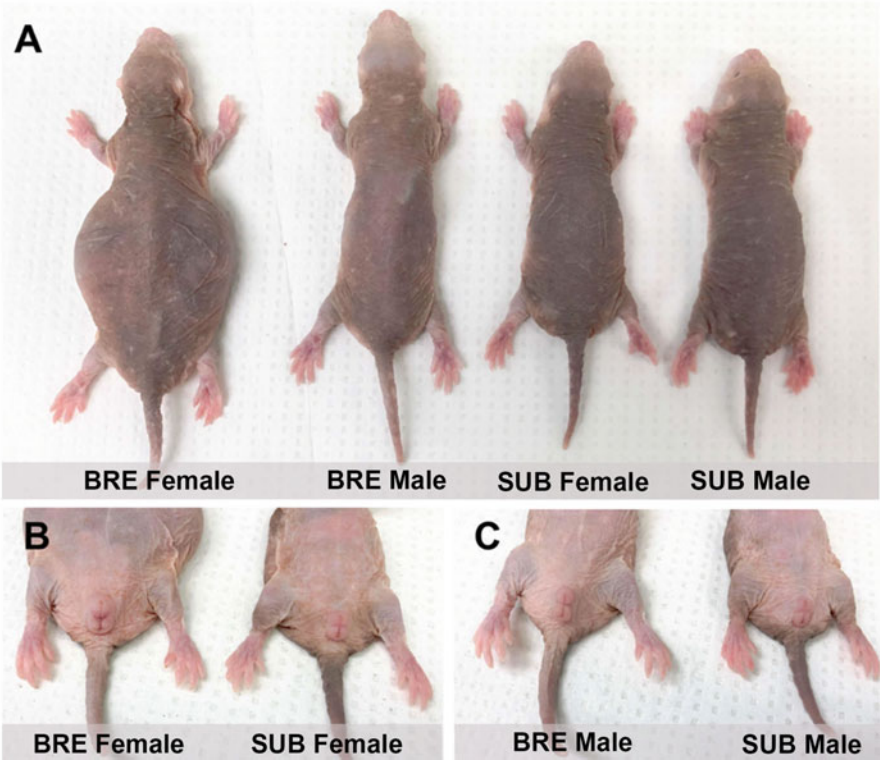

Fig. 2.5 Naked mole-rats show reduced sexual dimorphism compared to other mammals. (**a**) Breeders (BRE), particularly queens, are typically larger and heavier than subordinates (SUB). Subordinates are not sexually dimorphic in overall body size (**a**) or external genitalia (**b** and **c**). The genital mound enlarges in queens and she also develops a perforate vagina (**b**). The animals pictured here are all young to middle-aged adults ranging between 5 and10 years of age. Their weights are as follows: BRE female = 53.2 g, BRE male = 44.0 g, SUB female = 34.9 g, SUB male = 36.3 g

Thus, for some variables, social/pubertal status is a better predictor than sex. However, for other variables, the release from pubertal suppression seems to trigger the emergence of sex differences. Indeed, sex differences in behavior emerge post-puberty as both males and females begin to display sex-typical reproductive behaviors (e.g., mounting in males and lordosis in females). Urinary progesterone increases in females within days of removal from the colony and the vagina becomes perforate (Faulkes, Abbott, Jarvis, & Sherriff, 1990). As the female begins to breed, she will become longer as her vertebral column lengthens with each litter born (Dengler-Crish & Catania, 2007). Alternatively, the male endocrine transition is marked by an increase in urinary testosterone and, over time, breeding males often decrease in size. This emergence of sex differences extends to the nervous system where differences in gene and protein expression have been reported (Faykoo-Martinez et al., 2018; Holmes et al., 2008; Swift-Gallant et al., 2015; Zhou et al.,

2013). For example, female breeders have higher expression of estrogen receptor alpha and progesterone receptor mRNA and higher numbers of kisspeptin immunoreactive cells relative to male breeders and subordinates of both sexes (Swift-Gallant et al., 2015; Zhou et al., 2013), and these sex differences are all directly related to ovulation in female mammals. Not all emergent sex differences are in keeping with mammalian "norms," however. For example, the breeding female is socially dominant and the most aggressive individual in the colony (Clarke & Faulkes, 2001; Reeve, 1992), pushing and shoving other colony members. This is key as it reveals species-specific patterns of sex differences in brain and behavior as well as at least some dissociation between sex- and gender-typical variables.

All of this is consistent with the idea that sex is "less important" than social/pubertal status for sculpting brain and behavior in this species. More recently, however, we discovered that molecular sex differences exist in the brains of subordinates. Specifically, stress-related genes have higher expression in socially relevant brain regions (e.g., nucleus accumbens) in males compared to females (Faykoo-Martinez et al., 2018), suggesting that reproductive suppression is controlled, at least in part, in a sex-specific way. Thus, on the one hand, socially mediated pubertal suppression might prevent or delay the emergence of sex differences but, on the other, sex differences in mechanism might be critical for allowing pubertal suppression to exist in both sexes. That is to say, sex-specific mechanisms underlying reproductive suppression may serve to compensate for sex chromosomal gene expression, ultimately bringing males and females closer together on many variables (De Vries, 2004).

Studying diverse species like the naked mole-rat allows us to better understand the complex interplay between an organism's biological sex and sociosexual environment. From an evolutionary perspective, we learn about how species-specific social organization and reproductive strategy is associated with the type and magnitude of sex differences present in a species (e.g., sexual selection). From an organismal perspective, we learn about how social cues influence the development and maintenance of sex differences across the life span, which is hugely important for understanding individual differences in, and plasticity of, sex and gender variables. Employing both perspectives is necessary for understanding the causal relationship(s) between sex differences in brain and sex differences in behavior and can challenge how we think about sex and gender in both human and non-human animals.

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Spotlight references

Buffenstein, R. (2005). The naked mole-rat: A new long-living model for human aging research. *Journal Gerontology Series A: Biological Science and Medical Sciences, 60*, 1369–1377.

Clarke, F. M. & Faulkes, C. G. (1998). Hormonal and behavioural correlates of male dominance and reproductive status in captive colonies of the naked mole-rat, Heterocephalus glaber. *Proceedings of the Royal Society of London, Series B, 265*, 1391–1399.

Clarke, F. M. & Faulkes, C. G. (2001). Intracolony aggression in the eusocial naked mole-rat, Heterocephalus glaber. *Animal Behaviour, 61,* 311–324.

Dengler-Crish, C. M. & Catania, K. C. (2007). Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression. *Journal of Experimental Biology, 210,* 4351–4358.

De Vries, G. J. (2004). Minireview: Sex differences in adult and developing brains: Compensation, compensation, compensation. *Endocrinology*, *145*, 1063–1068.

Faulkes, C. G., Abbott, D. H. & Jarvis, J. U. M. (1990). Social suppression of ovarian cyclicity in captive and wild colonies of naked mole-rats, Heterocephalus glaber. *Journal of Reproduction & Infertility, 88*, 559–568.

Faulkes, C. G., Abbott, D. H., Jarvis, J. U. M. & Sherriff, F. E. (1990). LH responses of female naked mole-rats, Heterocephalus glaber, to single and multiple doses of exogenous GnRH. *Journal of Reproduction & Infertility, 89,* 317–323.

Faykoo-Martinez, M., Monks, D. A., Zovkic, I. B. & Holmes, M. M. (2018). Sex- and brain region-specific patterns of gene expression associated with socially-mediated puberty in a eusocial mammal. *PLoS One, 13*, e0193417. doi: 10.1371/journal.pone.0193417

Holmes, M. M., Goldman, B. D., Goldman, S. L., Seney, M. L. & Forger, N. G. (2009). Neuroendocrinology and sexual differentiation in eusocial mammals. *Frontiers in Neuroendocrinology, 30*, 519–533.

Holmes, M. M., Goldman, B. D. & Forger, N. G. (2008). Social status and sex independently influence androgen receptor expression in the eusocial naked mole-rat brain. *Hormones and Behavior, 54*, 278–285.

Holmes, M. M., Rosen, G. J., Jordan, C. L., de Vries, G. J., Goldman, B. D., & Forger, N. G. (2007). Social control of brain morphology in a eusocial mammal. *Proceedings of the National Academy of Sciences of the United States of America, 104*(25), 10548–10552.

Jarvis, J. U. M. (1981). Eusociality in a mammal: Cooperative breeding in naked mole-rat colonies. *Science, 212*(4494), 571–573.

Lacey, E. A. & Sherman, P. W. (1991). The biology of the naked mole-rat. In P. W. Sherman, J. U. M. Jarvis & R. D. Alexander (Eds.), *Social Organization of Naked Mole-Rat Colonies: Evidence for Divisions of Labour* (pp. 275–336). New Jersey: Princeton University Press.

Mooney, S. J., Filice, D. C. S., Douglas, N. R. & Holmes, M. M. (2015). Task specialization and task switching in eusocial mammals. *Animal Behavior, 109*, 227–233.

Peragine, D. E., Pokarowski, M., Mendoza-Viveros, L., Swift-Gallant, A., Cheng, H. -Y. M., Bentley, G. E., Holmes, M. M. (2017). RFamide-related peptide-3 (RFRP-3) suppresses sexual maturation in a eusocial mammal. *Proceedings of the National Academy of Sciences of the United States of America, 114,* 1207–1212.

Peroulakis, M. E., Goldman, B. & Forger, N. G. (2002). Perineal muscles and motoneurons are sexually monomorphic in the naked mole-rat (Heterocephalus glaber). *Journal of Neurobiology, 51,* 33–42.

Reeve, H. K. (1992). Queen activation of lazy workers in colonies of the eusocial naked mole-rat. *Nature, 358,* 147-149.

Seney, M. L., Kelly, D. A., Goldman, B. D., Šumbera, R. & Forger, N. G. (2009). Social structure predicts genital morphology in African mole-rats. *PLoS One, 4*, e7477. doi: 10.1371/journal.pone.0007477

Swift-Gallant, A., Mo, K., Peragine, D. E., Monks, D. A. & Holmes, M. M. (2015). Removal of reproductive suppression reveals latent sex differences in brain steroid hormone receptors in naked mole-rats, Heterocephalus glaber. *Biology of Sex Differences, 6,* 31.

Zhou, S., Holmes, M. M., Forger, N. G., Goldman, B. D., Lovern, M. B., Caraty, A., Kallo, I., Faulkes, C. G., & Coen, C. W. (2013). Socially regulated reproductive development: Analysis of GnRH-1 and kisspeptin neuronal systems in cooperatively breeding naked mole-rats (Heterocephalus glaber). *Journal of Comparative Neurology, 521*, 3003–3029.

References

Alexander, G. M., & Hines, M. (2002). Sex differences in response to children's toys in nonhuman primates (Cercopithecus aethiops sabaeus). *Evolution and Human Behavior, 23*(6), 467–479.

Altmann, S. A. (1962). A field study of the sociobiology of rhesus monkeys, Macaca mulatta. *Annals of the New York Academy of Sciences, 102*(2), 338–345.

Arnold, A. P., & Breedlove, S. M. (1985). Organizational and activational effects of sex steroids on brain and behavior: A reanalysis. *Hormones and Behavior, 19*(4), 469–498.

Berenbaum, S. A., & Hines, M. (1992). Early androgens are related to childhood sex-typed toy preferences. *Psychological Science, 3*(3), 203–206.

Bourgeois, J. P., Goldman-Rakic, P. S., & Rakic, P. (1994). Synaptogenesis in the prefrontal cortex of rhesus monkeys. *Cerebral Cortex, 4*(1), 78–96.

Bouvattier, C., Maione, L., Bouligand, J., Dode, C., Guiochon-Mantel, A., & Young, J. (2011). Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. *Nature Reviews Endocrinology, 8*(3), 172–182.

Brown, G. R., & Dixson, A. F. (1999). Investigation of the role of postnatal testosterone in the expression of sex differences in behavior in infant rhesus macaques (Macaca mulatta). *Hormones and Behavior, 35*(2), 186–194.

Corbier, P., Edwards, D. A., & Roffi, J. (1992). The neonatal testosterone surge: A comparative study. *Archives Internationales de Physiologie, de Biochimie et de Biophysique, 100*(2), 127–131.

Eaton, G. G., Worlein, J. M., & Glick, B. B. (1990). Sex differences in Japanese macaques (Macaca fuscata): Effects of prenatal testosterone on juvenile social behavior. *Hormones and Behavior, 24*(2), 270–283.

Eisler, J. A., Tannenbaum, P. L., Mann, D. R., & Wallen, K. (1993). Neonatal testicular suppression with a GnRH agonist in rhesus monkeys: Effects on adult endocrine function and behavior. *Hormones and Behavior, 27*(4), 551–567.

Ellinwood, W., Baughman, W., & Resko, J. (1982). The effects of gonadectomy and testosterone treatment in luteinizing hormone secretion in fetal rhesus monkeys. *Endocrinology, 110*, 183–189.

Feldman, S. S., Nash, S. C., & Cutrona, C. (1977). Influence of age and sex on responsiveness to babies. *Developmental Psychology, 13*(6), 675–676.

Fitch, R. H., Cowell, P. E., Schrott, L. M., & Denenberg, V. H. (1991). Corpus callosum: Demasculinization via perinatal anti-androgen. *International Journal of Developmental Neuroscience, 9*(1), 35–38.

Fitch, R. H., & Denenberg, V. H. (1998). A role for ovarian hormones in sexual differentiation of the brain. *Behavioral and Brain Sciences, 21*(3), 311–352.

Forest, M. G. (1979). Plasma androgens (testosterone and 4-androstenedione) and 17-hydroxyprogesterone in the neonatal, prepubertal and peripubertal periods in the human and the rat: Differences between species. *Journal of Steroid Biochemistry, 11*, 543–548.

Forest, M. G., & Cathiard, A. M. (1975). Pattern of plasma testosterone and delta4-androstenedione in normal newborns: Evidence for testicular activity at birth. *Journal of Clinical Endocrinology and Metabolism, 41*, 977–980.

Gaillard, J. M., Pontier, D., Allaine, D., Loison, A., Herve, J. C., & Heizmann, A. (1997). Variation in growth form and precocity at birth in eutherian mammals. *Proceedings of the Royal Society B: Biological Sciences, 264*(1383), 859–868.

Goldfoot, D. A., & van der Werff ten Bosch, J. J. (1975). Mounting behavior of female guinea pigs after prenatal and adult administration of the propionates of testosterone, dihydrotestosterone, and androstanediol. *Hormones and Behavior, 6*(2), 139–148.

Goy, R. W. (1970). Experimental control of psychosexuality. *Philosophical Transactions of the Royal Society B Biological Sciences, 259*(828), 149–162.

Goy, R. W. (1981). Differentiation of male social traits in female rhesus macaques by prenatal treatment with androgens: Variation in type of androgen, duration, and timing of treatment. In M. J. Novy & J. A. Resko (Eds.), *Fetal endocrinology* (pp. 319–339). Academic Press.

Goy, R. W., Bercovitch, F. B., & McBrair, M. C. (1988). Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. *Hormones and Behavior, 22*(4), 552–571.

Goy, R. W., & Deputte, B. L. (1996). The effects of diethylstilbestrol (des) before birth on the development of masculine behavior in juvenile female rhesus monkeys. *Hormones and Behavior, 30*(4), 379–386.

Goy, R. W., & McEwen, B. S. (1980). *Sexual differentiation of the brain*. MIT Press.

Goy, R. W., & Phoenix, C. H. (1972). The effects of testosterone propionate administered before birth on the development of behavior in genetic female rhesus monkeys. *UCLA Forum in Medical Sciences, 15*, 193–201.

Goy, R. W., & Resko, J. A. (1972). Gonadal hormones and behavior of normal and pseudohermaphroditic nonhuman female primates. *Recent Progress in Hormone Research, 28*, 707–733.

Goy, R. W., & Wallen, K. (1979). Experiential variables influencing play, foot-clasp mounting and adult sexual competence in male rhesus monkeys. *Psychoneuroendocrinology, 4*(1), 1–12.

Granger, B., Tekaia, F., Le Sourd, A. M., Rakic, P., & Bourgeois, J. P. (1995). Tempo of neurogenesis and synaptogenesis in the primate cingulate mesocortex: Comparison with the neocortex. *Journal of Comparative Neurology, 360*(2), 363–376.

Grattan, D. R., Rocca, M. S., Sagrillo, C. A., McCarthy, M. M., & Selmanoff, M. (1996). Antiandrogen microimplants into the rostral medial preoptic area decrease gamma-aminobutyric acidergic neuronal activity and increase luteinizing hormone secretion in the intact male rat. *Endocrinology, 137*(10), 4167–4173.

Harlow, H. F. (1962). The heterosexual affectional system in monkeys. *American Psychologist, 17*, 1–9.

Harlow, H. F. (1965). Sexual behavior in the rhesus monkey. In F. A. Beach (Ed.), *Sex and behavior* (pp. 178–192). Krieger.

Harlow, H. F., & Harlow, M. (1962). The effect of rearing conditions on behavior. *Bulletin of the Menninger Clinic, 26*, 213–224.

Harlow, H. F., & Lauersdorf, H. E. (1974). Sex differences in passion and play. *Perspectives in Biology and Medicine, 17*(3), 348–360.

Hassett, J. M., Rupp, H. A., & Wallen, K. (2010). Social segregation in male, but not female yearling rhesus macaques (Macaca mulatta). *American Journal of Primatology, 72*(2), 87–92.

Hassett, J. M., Siebert, E., & Wallen, K. (2008). Sex differences in rhesus monkey toy preferences parallel those of children. *Hormones and Behavior, 54*(3), 359–364.

Herman, R. A., Jones, B., Mann, D. R., & Wallen, K. (2000). Timing of prenatal androgen exposure: Anatomical and endocrine effects on juvenile male and female rhesus monkeys. *Hormones and Behavior, 38*, 52–66.

Herman, R. A., Measday, M. A., & Wallen, K. (2003). Sex differences in interest in infants in juvenile rhesus monkeys: Relationship to prenatal androgen. *Hormones and Behavior, 43*(5), 573–583.

Hines, M., Alsum, P., Roy, M., Gorski, R. A., & Goy, R. W. (1987). Estrogenic contributions to sexual differentiation in the female guinea pig: Influences of diethylstilbestrol and tamoxifen on neural, behavioral, and ovarian development. *Hormones and Behavior, 21*(3), 402–417.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology, 387*, 167–178.

Lovejoy, J., & Wallen, K. (1988). Sexually dimorphic behavior in group-housed rhesus monkeys (Macaca mulatta) at 1 year of age. *Psychobiology, 16*, 348–356.

Maestripieri, D., & Pelka, S. (2002). Sex differences in interest in infants across the lifespan – A biological adaptation for parenting? *Human Nature: An Interdisciplinary Biosocial Perspective, 13*(3), 327–344.

Maestripieri, D., & Wallen, K. (1995). Interest in infants varies with reproductive condition in group-living female pigtail macaques (Macaca nemestrina). *Physiology & Behavior, 57*(2), 353–358.

Maestripieri, D., & Zehr, J. L. (1998). Maternal responsiveness increases during pregnancy and after estrogen treatment in macaques. *Hormones and Behavior, 34*(3), 223–230.

Mann, D. R., Akinbami, M. A., Gould, K. G., Paul, K., & Wallen, K. (1998). Sexual maturation in male rhesus monkeys: Importance of neonatal testosterone exposure and social rank. *Journal of Endocrinology, 156*(3), 493–501.

Mann, D. R., Akinbami, M. A., Gould, K. G., Tanner, J. M., & Wallen, K. (1993). Neonatal treatment of male monkeys with a gonadotropin-releasing hormone agonist alters differentiation of central nervous system centers that regulate sexual and skeletal development. *Journal of Clinical Endocrinology and Metabolism, 76*(5), 1319–1324.

Mann, D. R., Davis-DaSilva, M., Wallen, K., Coan, P., Evans, D. E., & Collins, D. C. (1984). Blockade of neonatal activation of the pituitary-testicular axis with continuous administration of a gonadotropin-releasing hormone agonist in male rhesus monkeys. *Journal of Clinical Endocrinology and Metabolism, 59*(2), 207–211.

Martin, C. L., & Little, J. K. (1990). The relation of gender understanding to children's sex-typed preferences and gender stereotypes. *Child Development, 61*, 1427–1439.

Matsumoto, A. (1991). Synaptogenic action of sex steroids in developing and adult neuroendocrine brain. *Psychoneuroendocrinology, 16*, 25–40.

Meyer-Bahlburg, H. F., Dolezal, C., Baker, S. W., Carlson, A. D., Obeid, J. S., & New, M. I. (2004). Prenatal androgenization affects gender-related behavior but not gender identity in 5-12-year-old girls with congenital adrenal hyperplasia. *Archives of Sexual Behavior, 33*, 97–104.

Nevison, C. M., Brown, G. R., & Dixson, A. F. (1997). Effects of altering testosterone in early infancy on social behaviour in captive yearling rhesus monkeys. *Physiology & Behavior, 62*(6), 1397–1403.

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology, 65*, 369–382.

Phoenix, C. H., Jensen, J. N., & Chambers, K. C. (1983). Female sexual behavior displayed by androgenized female rhesus macaques. *Hormones and Behavior, 17*(2), 146–151.

Plant, T. M. (1986). A striking sex difference in the gonadotropin response to gonadectomy during infantile development in the rhesus monkey (Macaca mulatta). *Endocrinology, 119*(2), 539–545.

Pomerantz, S. M., Goy, R. W., & Roy, M. M. (1986). Expression of male-typical behavior in adult female pseudohermaphroditic rhesus: Comparisons with normal males and neonatally gonadectomized males and females. *Hormones and Behavior, 20*(4), 483–500.

Pomerantz, S. M., Roy, M. M., Thornton, J. E., & Goy, R. W. (1985). Expression of adult female patterns of sexual behavior by male, female, and pseudohermaphroditic female rhesus monkeys. *Biology of Reproduction, 33*(4), 878–889.

Rakic, P. (1988). Specification of cerebral cortical areas. *Science, 241*, 170–176.

Resko, J. A. (1985). Gonadal hormones during sexual differentiation in vertebrates. In N. Adler, D. Pfaff, & R. W. Goy (Eds.), *Handbook of behavioral neurobiology* (pp. 21–42). Plenum Press.

Resko, J. A., Buhl, A. E., & Phoenix, C. H. (1987). Treatment of pregnant rhesus macaques with testosterone propionate: Observations on its fate in the fetus. *Biology of Reproduction, 37*(5), 1185–1191.

Resko, J. A., & Ellinwood, W. E. (1981). Testicular hormone production in fetal rhesus macaques. In M. J. Novy & J. A. Resko (Eds.), *Fetal endocrinology* (pp. 253–267). Academic Press.

Resko, J. A., Ellinwood, W. E., Pasztor, L. M., & Huhl, A. E. (1980). Sex steroids in the umbilical circulation of fetal rhesus monkeys from the time of gonadal differentiation. *Journal of Clinical Endocrinology and Metabolism, 50*(5), 900–905.

Singh, S. M., Gauthier, S., & Labrie, F. (2000). Androgen receptor antagonists (antiandrogens): Structure-activity relationships. *Current Medicinal Chemistry, 7*(2), 211–247.

Sodersten, P., Gray, G., Damassa, D. A., Smith, E. R., & Davidson, J. M. (1975). Effects of a non-steroidal antiandrogen on sexual behavior and pituitary-gonadal function in the male rat. *Endocrinology, 97*(6), 1468–1475.

Thornton, J., & Goy, R. W. (1986). Female-typical sexual behavior of rhesus and defeminization by androgens given prenatally. *Hormones and Behavior, 20*(2), 129–147.

Thornton, J., Zehr, J. L., & Loose, M. D. (2009). Effects of prenatal androgens on rhesus monkeys: A model system to explore the organizational hypothesis in primates. *Hormones and Behavior, 55*, 633–645.

Tomaszycki, M. L., Davis, J. E., Gouzoules, H., & Wallen, K. (2001). Sex differences in infant rhesus macaque separation-rejection vocalizations and effects of prenatal androgens. *Hormones and Behavior, 39*(4), 267–276.

Veldhuis, J. D., Urban, R. J., & Dufau, M. L. (1992). Evidence that androgen negative feedback regulates hypothalamic gonadotropin-releasing hormone impulse strength and the burst-like secretion of biologically active luteinizing hormone in men. *Journal of Clinical Endocrinology and Metabolism, 74*(6), 1227–1235.

Wallen, K. (1996). Nature needs nurture: The interaction of hormonal and social influences on the development of behavioral sex differences in rhesus monkeys. *Hormones and Behavior, 30*(4), 364–378.

Wallen, K. (2001). Sex and context: Hormones and primate sexual motivation. *Hormones and Behavior, 40*(2), 339–357.

Wallen, K. (2005). Hormonal influences on sexually differentiated behavior in nonhuman primates. *Frontiers in Neuroendocrinology, 26*(1), 7–26.

Wallen, K. (2009). Sexual differentiation of behavior in nonhuman primates. In D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, & R. T. Rubin (Eds.), *Hormones, brain and behavior* (2nd ed., pp. 1843–1868). Elsevier.

Wallen, K. (2017). Sexual differentiation of behavior in nonhuman primates. In D. W. Pfaff & M. Joels (Eds.), *Hormones, brain and behavior* (3rd ed., pp. 225–245). Elsevier.

Wallen, K., & Baum, M. J. (2002). Masculinization and defeminization in altricial and precocial mammals: Comparative aspects of steroid hormone action. In D. W. Pfaff, A. P. Arnold, S. E. Fahrbach, A. M. Etgen, & R. T. Rubin (Eds.), *Hormones, brain and behavior* (pp. 385–423). Elsevier.

Wallen, K., Bielert, C., & Slimp, J. (1977). Foot clasp mounting in the prepubertal rhesus monkey: Social and hormonal influences. In F. E. Poirie & S. Cevalier-Skolnikoff (Eds.), *Biosocial development among primates* (pp. 439–461). Garland Publishing.

Wallen, K., Goldfoot, D., & Goy, R. W. (1981). Peer and maternal influences on the expression of foot-clasp mounting by juvenile male rhesus monkeys. *Developmental Psychology, 14*, 299–309.

Wallen, K., Maestripieri, D., & Mann, D. R. (1995). Effects of neonatal testicular suppression with a GnRH antagonist on social behavior in group-living juvenile rhesus monkeys. *Hormones and Behavior, 29*(3), 322–337.

Wallen, K., Winston, L. A., Gaventa, S., Davis-DaSilva, M., & Collins, D. C. (1984). Periovulatory changes in female sexual behavior and patterns of ovarian steroid secretion in group-living rhesus monkeys. *Hormones and Behavior, 18*(4), 431–450.

Whalen, R. (1968). Differentiation of neural mechanisms which control gonadotropin secretion and sexual behavior. In M. Diamond (Ed.), *Perspectives in reproduction and sexual behavior* (pp. 303–340). Indiana Press.

Zehr, J. L., Tannenbaum, P. L., Jones, B., & Wallen, K. (2000). Peak occurrence of female sexual initiation predicts day of conception in rhesus monkeys (Macaca mulatta). *Reproduction, Fertility and Development, 12*, 397–340.