Chapter 5
Prenatal Androgens Affect Development and Behavior in Primates

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Abbreviations for Terminology

CAH	Congenital adrenal hyperplasia
CAIS	Complete androgen insensitivity syndromes
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
PAIS	Partial androgen insensitivity syndromes
PCOS	Polycystic ovary syndrome
TP	Testosterone propionate
ZFX	X-linked zinc finger protein gene
ZFY	Y-linked zinc finger protein gene

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5.1 Introduction

Maturation and differentiation persists throughout childhood, adolescence, and adult life into senescence; however, the foundation for normal postnatal development is established during fetal ontogeny. The organizational hypothesis proposes that nongenomic, environmental factors within the intrauterine environment attribute to prenatal programming (Phoenix et al. 1959). From the inception of this hypothesis over 50 years ago, researchers have documented the effects of exposure to prenatal steroid hormones, particularly androgens, on the behavior and growth of primate offspring. The effects of androgen within the intrauterine environment from maternal and exogenous sources can be observed in long-term changes to multiple developmental trajectories, including somatic growth, homeostatic functions of the body, and differentiation of sex-typical morphology, physiology, and behavior. Thus, the sensitivity and plasticity of the fetus during development toward androgens and other physiological cues from the mother and environment may underlie the development of diseases, a premise postulated by the Barker hypothesis (Barker 1998). In the current chapter, we discuss a number of prenatal and postnatal developmental outcomes associated with exposure to normal variations and excessive concentrations of androgens during prenatal life in human and nonhuman primates. In addition, it seems that the timing of androgen exposure during gestation and the sex of the fetus are two major factors that contribute to the concentration of androgens in the prenatal environment and the ultimate outcomes. Therefore, we also discuss the timing of androgen exposure during gestation and the sex of the fetus as modulating variables on the androgen-induced effects on development.

5.2 The Endogenous Origins of Androgens During Fetal Development

The preponderance of evidence outlines three periods in primate lifespan in which there are significant fluctuations in androgen production: intrauterine and infant development, adolescence and puberty, and senescence [e.g., human (*Homo sapiens sapiens*) (Forest 1983; Rabinovici and Jaffe 1990; Seidman 2003; Richmond and Rogol 2007; Seidman 2007), western lowland gorilla (*Gorilla gorilla gorilla*) (Stoinski et al. 2002), rhesus macaque (*Macaca mulatta*) (Resko and Roselli 1997; Mann et al. 1998), crab-eating macaque (*Macaca fascicularis*) (Steiner and Bremner 1981), chimpanzee (*Pan troglodytes*) (Marson et al. 1991; Martin et al. 1977), common marmoset (*Callithrix jacchus*) (Abbott and Hearn 1978b; Dixson 1986), Geoffroy's tufted-ear marmoset (*Callithrix geoffroyi*) (Birnie et al. 2011), cotton-top tamarin (*Saguinus oedipus*) (Ginther et al. 2002), gray mouse lemur (*Microcebus murinus*) (Aujard and Perret 1998)]. However, it is during fetal development that the organizational effects of androgens are most observed, e.g., affecting the onset of other critical periods such as puberty (Jones and Verkauf 1971; Goy et al. 1988b; Zehr et al. 2005; Herman et al. 2006) and androgen sensitivity

over the lifespan (Resko and Ellinwood 1984; Dumesic et al. 1997; Eisner et al. 2002; Abbott et al. 2008). The prenatal androgen milieu that primate fetuses are exposed to in the intrauterine environment originates from the fetus or mother, endogenously, or from environmental sources (see Svechnikov et al. 2010).

5.2.1 Fetal Origins of Androgen

The ontogenesis of fetally derived androgen in human and nonhuman primates is derived from the gonads or adrenal gland (d'Aux and Murphy 1974; Huhtaniemi 1994; Resko and Roselli 1997; Svechnikov and Söder 2008; Rouiller-Fabre et al. 2009). The primate fetal endocrine system develops early in gestation and results in androgen production within the first gestational trimester (Rabinovici and Jaffe 1990). In the presence of gene products from two loci—the *Sry* gene located on the Y chromosome and the *Sox9* gene on the X chromosome—the undifferentiated embryonic gonad develops into testes, occurring as soon as gestational week 8 in humans (Huhtaniemi 1994; Bendsen et al. 2003; O'Shaughnessy et al. 2007). The number of male human fetal Leydig cells increases substantially until peaking in week 19 of gestation, contemporaneously with a peak in the concentration of testosterone and dihydrotestosterone (DHT) in the blood and testicular tissue in the fetus (Tapanainen et al. 1981; Rabinovici and Jaffe 1990; Svechnikov and Söder 2008). Similar to human fetal development, male nonhuman primates begin to synthesize and secrete androgens shortly after the external genitalia and internal reproductive anatomy begin to virilize. For example, the external genitalia of rhesus monkey differentiate between gestational days 38 and 40 during the 168-day gestation (van Wagenen and Simpson 1965; Resko 1985), and fetal androgen production begins between gestational days 35 and 50 (Resko and Ellinwood 1981) and peaks around gestational days 40–75 (Resko 1985). Conventional wisdom suggests that, in contrast to male primate gonads, female fetal ovaries do not undergo significant steroidogenesis and the hypothalamic–pituitary–gonadal axis has been thought to remain quiescent prenatally (d'Aux and Murphy 1974; Ellinwood et al. 1982; Rabinovici and Jaffe 1990). However, recent evidence indicates that human fetal ovaries may express the androgen biosynthetic enzyme CYP17 and androgen receptors by midgestation (Cole et al. 2006; Fowler et al. 2011). Thus, primate fetal ovaries may not be as quiescent as traditionally viewed as the expression of such components suggests that ovaries have the capacity to produce androgens and be responsive to them.

In addition to the gonadal androgens, the primate adrenal cortex synthesizes and secretes androgens, mainly dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), in the fetal adrenocortical zone, an inner zone of the fetal adrenal gland present only in primates and some edentata (Conley et al. 2004; Pattison et al. 2005; Abbott and Bird 2009). The fetal zone is developed by midgestation and begins producing androgens, then regresses shortly after birth coinciding with a decrease in adrenal androgens in human and nonhuman primates (McNulty et al. 1981;

Mapes et al. 2002; Pattison et al. 2005, reviewed by Mesiano and Jaffe 1997). Unlike fetal gonadal androgen production that only occurs in males, the fetal zone in the adrenal cortex develops and secretes adrenal androgens in both sexes.

5.2.2 Maternal Origins of Androgen

Primate pregnancies are typified by a substantial increase in the production of progesterone and estrogen derived from ovarian and placental sources (Albrecht and Pepe 1990; Ojeda 2004). From conception, there is also a significant rise in maternal androgen in human and nonhuman primates [e.g., human (Rivarola et al. 1968; Bammann et al. 1980; Castracane and Asch 1995; Castracane et al. 1998), bonnet monkey (*Macaca radiata*) (Rao and Kotagi 1983), olive baboon (*Papio anubis*) (Castracane and Goldzieher 1983; Hodges et al. 1984), rhesus macaque (Challis et al. 1975; Ellinwood et al. 1989), marmoset (Chambers and Hearn 1979; Fite et al. 2005; French et al. 2010; Smith et al. 2010)]. While the total testosterone levels increase from conception, free testosterone levels remain similar to non-pregnancy levels until week 28 of gestation in humans. This suggests that much of the change in total testosterone is associated with an increase in bound testosterone. In fact, there is a significant increase in sex-hormone-binding globulin levels and a decrease in testosterone metabolism from the beginning of pregnancy (Saez et al. 1972; Bammann et al. 1980). In contrast, although maternal adrenal androgen production increases during pregnancies (Gant et al. 1971), there is a net decrease in circulating maternal adrenal androgen (Milewich et al. 1978), primarily due to the conversion of DHEAS into estrogen by the placenta (Siiteri and MacDonald 1963). While the primate placenta is critical in the production of gestational progesterones, estrogens, and peptide hormones such as chorionic gonadotrophin, corticotrophin-releasing hormone, and the insulin-like growth factors (reviewed by Pepe and Albrecht 1995; Murphy et al. 2006), it does not produce androgens (e.g., human: Licht et al. 1998; rhesus monkey: Ellinwood et al. 1989). Therefore, the rise in circulating androgens in pregnant women and other female primates originates from either the fetus or the mother.

Currently, there is some inconsistency in research that evaluates whether fetally derived androgens enter maternal circulation and consequently contribute to the elevated maternal androgens during gestations in primate species. In humans, fetal testosterone in both male and female fetuses is positively associated with maternal testosterone in mid- to late gestation, that is, 15–38 weeks gestational age (Gitau et al. 2005). While adult female adrenal glands and ovaries clearly produce androgens (Burger 2002; Stanczyk 2006), some studies have documented a significant increase in maternal androgens when carrying a male fetus as opposed to a female fetus in pregnant women (Nagamani et al. 1979; Meulenberg and Hofman 1991) and other female primates [yellow baboon (*Papio cynocephalus*) (Altmann et al. 2004), red-fronted lemur (*Eulemur fulvus rufus*) (Ostner et al. 2003)]. Antithetically, other studies do not report any effect of male fetuses on maternal serum androgens,

even when some of these studies report a clear increase in amniotic fluid and umbilical cord blood androgens when male fetuses are present (Rivarola et al. 1968; Glass and Klein 1981; Rodeck et al. 1985; Atkinson et al. 1996; Sir-Petermann et al. 2002; Steier et al. 2002; Troisi et al. 2003; van de Beek et al. 2004; French et al. 2010). Beyond fetal sex, it has been noted that androgens significantly increase in maternal circulation in human and nonhuman primates shortly after conception and before virilization of fetal gonads—a developmental step that precedes fetal gonadal steroidogenesis which occurs around week 8 in human gestation—or even the implantation and elaboration of the fetoplacental unit. In addition, subcutaneous injections of testosterone propionate (TP) into pregnant rhesus monkeys result in an increase in testosterone and androstenedione, but not estradiol or estrone, in maternal and fetal serum (Abbott et al. 2008). Therefore, while it is not clear if fetal androgens contribute to circulating androgens in the maternal system during mid- to late gestation, it is apparent that the initial rise in maternal androgens and, to some degree, later maternal androgens as well as fetal androgens can be attributed to the maternal endocrine system (Reyes et al. 1973).

5.3 Prenatal Androgens and Genital Differentiation

The dynamics of mammalian sexual differentiation is one of the best-understood ontogenetic processes in integrative biology. Several excellent recent reviews have encapsulated this process (Morris et al. 2004; Wallen 2005; Arnold 2009), and we briefly summarized this process above (see Sect. 5.2.1). With the caveat that there are important direct genetic effects on the differentiation of morphology, brain, and behavior (e.g., Arnold et al. 2004; Arnold 2009), the pattern described above—*gene action → gonadal differentiation → endocrine consequence → phenotypic divergence*—characterizes the common mammalian process of sexual differentiation.

5.3.1 Prenatal Androgen Excess on Human Genital Differentiation: Clinical Studies

There are three classic human disorders that lend credence to the genetic–gonadal–endocrine cascade hypothesis for the differentiation of genital morphology in humans. In congenital adrenal hyperplasia (CAH) and 46,XX male syndrome, chromosomal females (46,XX) have mutations in the genes that regulate steroidogenesis. In addition to other alterations in steroid biosynthesis pathways, androgen concentrations in fetal circulation increase [CAH (White and Speiser 2000; White and Speiser 2000), 46,XX male syndrome (Kousta et al. 2010)]. Female infants with salt-wasting CAH are born with completely fused labia and the clitoris hypertrophies into a phallus (Braga and Pippi Salle 2009; Nimkarn and New 2010). At birth, individuals with 46,XX male syndrome present with small testes, a masculinized

phallus and scrotum, and an absence of Mullerian tissue (Vorona et al. 2007), all of which are markers of a complete masculinization of genitalia in these chromosomal females via excess prenatal androgen exposure.

Finally, there are a variety of genetic variants that are associated with the lack of masculinization of external genitalia in otherwise normal 46,XY chromosomal males (Wisniewski and Mazur 2009). In one variant, males have dysfunctional intracellular androgen receptors, leading to either partial or complete androgen insensitivity syndromes (PAIS and CAIS) (Wisniewski and Mazur 2009; Philibert et al. 2010). Since the androgen receptors do not respond normally when bound with androgens, the effects of the androgen–receptor complex on nuclear DNA transcription are altered or eliminated (Ahmed et al. 2000). Individuals that present with PAIS have varying degrees of genital ambiguity, depending on the severity of the insensitivity, while individuals with CAIS present with a complete female phenotype, including labia, a blind vagina, female-typical fat distribution, and female breasts (Werner et al. 2010). Other 46,XY syndromes involve mutations that alter enzymatic function in steroidogenesis such as 5α-reductase 2 deficiency—reducing testosterone conversion to DHT and feminizing external genitalia (Wilson et al. 1993)—and 17β-hydroxysteroid dehydrogenase 3 deficiency—impairing androstenedione conversion to testosterone and feminizing internal sex ducts and external genitalia (Boehmer et al. 1999; Wisniewski and Mazur 2009; George et al. 2010). In conditions associated with disrupted androgen signaling or biosynthesis, the results emphasize the central importance of the choreography among genes, gonads, and androgen production and sensitivity in regulating the differentiation of external genitalia in human beings.

5.3.2 Prenatal Androgen Excess on Nonhuman Primate Genital Differentiation: Two Models

5.3.2.1 Manipulation of Prenatal Androgens on Genital Differentiation in Rhesus Macaques

Among nonhuman primates, the processes that underlie the differentiation of external genitalia have been most exquisitely outlined for the rhesus macaque in Prahalada et al. (1997), Thornton et al. (2009), and briefly above (see Sect. 5.2.1). Treatment of pregnant mothers with supplemental exogenous androgen, or disruption of androgen biosynthesis via exogenous treatment, has been extensively employed—being reported as early as Young et al. (1964)—to assess the mechanisms of endocrine effects on genital structure, and the timing of this differentiation. The degree of female fetal genital virilization depends on the timing and dose of supplemental androgen provided to the pregnant mother. Exposure to daily doses of 610–750-mg TP for 25–50 days starting on gestational day 39 (early androgenized females) completely masculinized the external genitalia of genetic females, including complete scrotal and penile development and a lack of a vaginal orifice (Young et al. 1964;

Eaton et al. 1973). These results have been replicated with significantly lower doses of daily injections of testosterone (15 mg/day) and also with DHT (Goy 1981; Goy and Robinson 1982; cf. Herman et al. 2000). However, female fetuses exposed to the same treatment regimen and dose from gestational days 115 to 139 (late androgenized females) showed no permanent masculinization of external genitalia and had normal vaginal openings (Goy et al. 1988a; Herman et al. 2000). It appears, therefore, that the critical period for genital masculinization is early in fetal development, and late androgen manipulations have minimal effects on genital structure in macaque monkeys.

An alternative approach for assessing the role of steroid exposure on the sexual differentiation of genital structure is to pharmacologically block the effects of androgens or alter steroid biosynthesis in developing male fetuses. Finasteride is a compound that inhibits the enzyme 5α-reductase, which is critical for the bioconversion of testosterone to the potent androgen DHT. Male macaques whose mothers were treated with finasteride from days 20 to 100 of gestation displayed significantly smaller phalluses and small and poorly developed scrota, suggesting that DHT plays a role, along with testosterone, in masculinizing male genitalia (Prahalada et al. 1997). In a similar vein, Herman et al. (2000) treated pregnant mothers carrying male fetuses with the potent androgen receptor blocker flutamide either early in gestation (days 35–70) or late in gestation (days 115–155). There were again subtle but significant effects of flutamide treatment on some aspects of male genitalia. Males with either early or late treatment had significantly shorter phalluses, and their overall "masculinization" scores were lower than vehicle males, especially for the early gestational exposure males (Herman et al. 2000).

5.3.2.2 The Unique Intrauterine Environment of Marmoset Monkeys: Influences of Prenatal Androgen Exposure on Subsequent Genital Differentiation

Marmosets and tamarins constitute an interesting and important test case of sexual differentiation among primates. They are the only primates with obligate twinning, with dizygotic twin births constituting 70–90% of litters (Ross et al. 2007a). Unlike most twins, marmosets develop placental anastomoses between twins and share a common blood supply. This has important implications for genetic chimerism, as co-twins pass embryonic stem cells to each other and are genetic chimeras in multiple tissues, including germ line (Ross et al. 2007b). From the perspective of sexual differentiation, the intimate vascular connections between co-twins suggest the potential for interesting prenatal androgen effects on sexual differentiation, as males share a blood supply with their female co-twin. One might expect masculinization of female offspring in the case of male–female co-twins and the absence of masculinization in the case of female–female co-twins. As an evolutionary adaptation to prevent female masculinization, Abbott and Hearn suggested that the majority of sexual differentiation in marmosets may occur postnatally because of this shared fetal blood supply (Abbott and Hearn 1978a; Abbott 1984). This is supported by the

appearance of elevated testosterone levels in males from 15 to 100 days of age (Abbott and Hearn 1978a; Dixson 1986; Birnie et al. 2011). Postnatal testosterone treatment masculinized female marmoset genitalia, with hypertrophy of the clitoris and enlargement of the labia to resemble scrotal folds (Abbott and Hearn 1978b; Abbott 1984). Neonatal castration produced adult males with small genitalia, and testosterone treatment of these adults produced an increase in penile and scrotal size (Dixson 1993). Like other nonhuman primates (e.g., rhesus macaques, Brown et al. 1999), androgen exposure can further masculinize marmoset genitalia in the immediate postnatal period, but marmoset genitalia are already differentiated at birth (Abbott and Hearn 1978b).

Several reports have appeared on the occurrence of "ambiguous genitalia" in female marmosets. Isachenko et al. (2002) reported on a high incidence of fused labia in common marmosets (~32%) at the German Primate Center. Normally, the vulval opening in female marmosets is 8–10 mm, but in the affected females, the labial fold was only an average of 2.1 mm in length. Surgical separation of the fused labia revealed a normal vagina and cervix, and ultrasound confirmed the presence of a uterus and ovaries. In a case study, a female common marmoset that had ambiguous, masculinized genitalia also expressed the testis-determining *Sry* gene and the Y-linked zinc finger protein gene (ZFY) as measured in DNA analysis of hair bulb tissue (Sanchez-Morgado et al. 2003). A similar example of an XX/XY female lion tamarin (*Leontopithecus chrysomelas*) displaying masculinized external genitalia was recently reported (Goldschmidt et al. 2005). At the Callitrichid Research Center, we have recently genotyped two male and two female Wied's black-tufted-ear marmosets (*Callithrix kuhlii*) for X-linked zinc finger protein (ZFX) and ZFY (see Fig. 5.1). In the gels, both males clearly expressed ZFY. In contrast, the female with a female co-twin expressed only ZFX, while the female with a male co-twin expressed ZFY. The ZFY female also had ambiguous genitalia at birth that became more feminized at 6 months.

Clearly, unusual features are associated with the development of genital structures in marmosets and tamarins, and we hope to determine if some of these phenomena interact with prenatal and immediate postnatal steroids of maternal origin. Given that postnatal androgen can masculinize female genitalia (Abbott and Hearn 1978b), it appears that some of the variation in genitalia may be caused by androgen exposure, but the presence of Y chromosome and its genes in some females suggests a more complicated scenario. This phenomenon is similar to freemartinism in cattle and other species (Lillie 1917; Capel and Coveney 2004), with the important exception that XX/XY marmosets are not sterile. Future work in our lab will address the extent to which ambiguous female genitalia are systematically associated with the presence of Y chromosomes, and whether more subtle aspects of female reproduction (e.g., age at puberty, age at first litter, offspring survivability) are associated with having a male co-twin. However, without more invasive manipulations, such as administering androgen receptor blockers to female fetuses with male co-twins or selectively knocking down Y chromosome activity, it will be difficult to disentangle genetic from endocrine influences on the development of female genitalia.

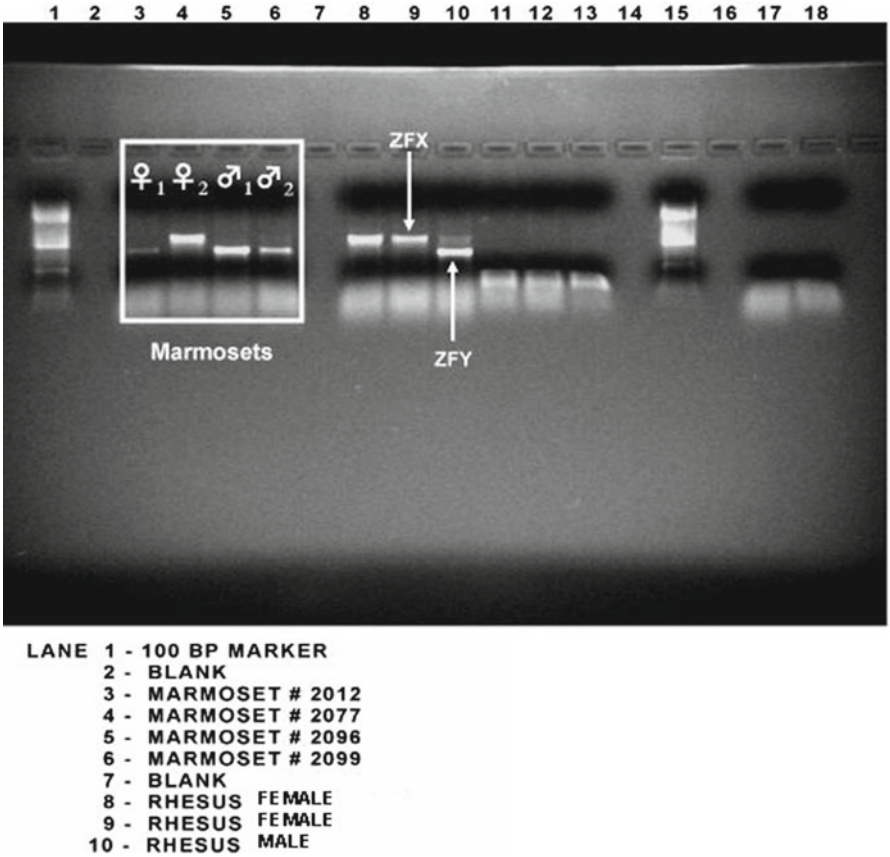

Fig. 5.1 Genotyping of marmosets for Y-chromosome and X-chromosome zinc finger protein (ZFY and ZFX, respectively). *Lanes 8 and 9* of the gel contain DNA from female rhesus macaques, while *lane 10* contains DNA from a male rhesus, revealing the sex-chromosome-specific patterns of zinc finger proteins. *Lanes 5 and 6* contain DNA from two male marmosets, and clear ZFY signals are evident. *Lane 4* contains DNA from a female marmoset that had shared the uterus with a female co-twin, and expresses ZFX. *Lane 3* contains DNA from a female marmoset that had shared the uterine environment with a male co-twin. She expresses ZFY, demonstrating that she maintains viable cells acquired early in embryonic development from her male co-twin

5.4 Prenatal Androgens and Fetal and Postnatal Growth

Androgens can also have organizational and activational effects on physical growth that manifest throughout the lifespan in primates. Androgens are potent anabolic hormones and, working in concert with growth hormones, can affect body growth. In pubertal development, testosterone can promote the growth of muscle mass and strength, increase bone density, and alter body composition,

specifically regional fat distribution (reviewed by Hiort 2002; Randall et al. 2002). In prenatal development, evidence of androgen-induced alteration of fetal growth and postnatal development can be observed in normal variation in maternal androgens and clinical cases of hyperandrogenism in pregnant women as well as primate models.

In normal pregnancies, maternal androgen production surges beginning at conception until about midgestation when levels are comparable to male conspecifics (see Sect. 5.2.2). In addition, there is normal variation in total androgen production that occurs between pregnancies in humans and nonhuman primates [e.g., human (Castracane et al. 1998); marmoset (Smith et al. 2010)]. This provides a source of androgen that fetuses may be exposed to in the intrauterine environment that would vary between pregnancies and, that is, more accessible to researchers than fetal or umbilical cord blood or amniotic fluid. From this research, it seems that the concentration of androgen present in maternal circulation may influence fetal growth. For example, early second trimester (gestation week 17) maternal testosterone was negatively correlated with birth weight and length in full-term human infants (Carlsen et al. 2006). Birth weight was 160 g lower in babies born to mothers with testosterone levels in the 75th percentile compared to those born to mothers with testosterone levels in the 25th percentile, a weight difference similar to the impact from fetal sex. In Geoffroy's tufted-ear marmoset monkeys, we have recently noted that male and female neonates born to mothers with high first trimester androgen levels had a smaller gross birth size (e.g., weighed less, were shorter, and had less upper-body girth) compared to marmosets born to mothers with low first trimester androgens (Smith et al. 2010). In addition, offspring from mothers with high first trimester androgens had depressed early infancy weight and girth gains and increased late infancy and juvenile growth rates in body girth. Further, maternal androgen levels in this species are not influenced by fetal sex ratio (French et al. 2010). Thus, maternally derived androgens early in gestation may alter normal fetal and subsequent prepubescent postnatal growth in primates.

The effects on physical growth do not seem to hold with late gestational androgens. Birth weight and length as well as first year gains are not associated with total or free androstenedione, testosterone, or DHT in maternal or umbilical cord blood collected at birth in full-term babies in humans (Gemer et al. 1997; Troisi et al. 2003; Whitehouse et al. 2010). Notably, Carlsen et al. (2006) also measured testosterone in maternal blood during gestation week 33 and found a negative correlation between third trimester maternal testosterone and birth weight and length of newborns. However, as gestational week 17 and 33 testosterone were highly correlated (Pearson's $r = 0.75$) in that study, the association between late gestation androgens and birth size may be an artifact of the association between testosterone throughout gestation. In our research with marmoset monkeys, maternal androgens in the second or third trimester were not associated with prenatal and postnatal growth in offspring (Smith et al. 2010). Additional work is required to substantiate these findings. However, these data support the contention that subtle changes in the early, rather than

late, gestational androgenic environment can influence fetal development and subsequent postnatal growth in primates.

5.4.1 *Prenatal Androgen Excess on Human Growth: Clinical Studies*

There are several conditions that lead to a hyperandrogenic state in women, but the effects of hyperandrogenism associated with polycystic ovary syndrome (PCOS) on fetal development have been the most extensively and systematically studied. Women with PCOS have higher total and free androgens during early gestation (Sir-Petermann et al. 2002; reviewed in Abbott et al. 2005; Ehrmann 2005; Xita and Tsatsoulis 2006). Interestingly, several studies have noted that fetal growth may be reduced if the mother has PCOS. For example, Sir-Petermann et al. (2005) noted that women with PCOS give birth to small-for-gestational-age newborns (12.8%) at a higher prevalence compared to the general population (2.8%) with no difference in the rate of large-for-gestational-age newborns. In a meta-analysis that included 12 studies evaluating the birth weight in infants born to women with or without PCOS, women with PCOS gave birth to newborns with lower birth weights compared to controls, though this was not replicated in a subset of the studies analyzed (Boomsma et al. 2006). However, this effect should not be overstated as other studies have failed to replicate these results (e.g., Anderson et al. 2010). It is not clear why some studies report an effect on fetal growth while others lack support. Interestingly, while hyperandrogenism is used as a diagnostic criteria for women with PCOS, fetuses from women with PCOS may not always be exposed to intrauterine androgen excess during development. This is evident when comparing results from two recent studies in which one reported hyperandrogenism in the umbilicical cord blood during pregnancies in women with PCOS (Barry et al. 2010) but another did not (Anderson et al. 2010). If hyperandrogenism in the intrauterine environment is required to alter fetal growth, this may be one reason why only subsets of women with PCOS give birth to small-for-gestational-age newborns, and some studies do not report a difference in the birth size of babies born to women with PCOS compared to the general population.

Exposure to androgen excess during gestation can cause an increase in weight gain in adolescence and adulthood, particularly in adipose tissue, and lead to an increased incidence of obesity. In this regard, approximately 63% of adolescent girls with PCOS were considered obese compared to 32% of normal girls of similar age and ethnic background in a United States population (Coviello et al. 2006). In addition, girls that present with precocious pubarche with functional ovarian hyperandrogenism are more commonly born with small-for-gestational-age birth weights compared to girls with precocious pubarche without functional ovarian hyperandrogenism (Ibanez et al. 1998). Thus, exposure to androgen excess via maternal or fetal hyperandrogenism seems to alter fetal programming resulting in an abnormal growth trajectory that persists into adulthood.

5.4.2 Prenatal Androgen Excess on Nonhuman Primate Growth: The Rhesus Model

Rhesus monkeys have been used to develop a nonhuman primate model of PCOS. In the Wisconsin National Primate Research Center, a population of adult female rhesus monkeys receive androgen treatments during pregnancies (for additional general methods, see Sect. 5.3.2.1, Abbott et al. 2005) that results in a significant elevation of androgens in fetal circulation (Resko et al. 1987; Abbott et al. 2008). From this research program, it has been documented that early prenatal androgens may affect male fetal growth but not female fetal growth (Herman et al. 2000; Abbott et al. 2008), although recent evidence indicates a modest increase in head circumference in female fetuses (Abbott et al. 2010). However, early gestational exposure to androgen excess seems to pronouncedly affect postnatal growth in female rhesus monkeys. Female infants exposed to early testosterone treatment exhibit a heavier body weight (~10%) at 2 months of age compared to controls (Abbott et al. 2009, 2010). Prenatal testosterone treatment did not alter body weight or girth in male neonates during the first week of life (Bruns et al. 2004). However, this null effect in males may be a function of the low sample size or not treating during a critical period in fetal development as testosterone treatments were administered at early, mid-, or late gestation for different subjects. In addition, female rhesus monkeys exposed to testosterone excess early in gestation have increased body weight at menarche (Goy and Robinson 1982) and total body mass (Kemnitz et al. 1988), abdominal skinfold thickness (Abbott et al. 1998), and total abdominal and intra-abdominal fat depots that are independent of total body adiposity (Eisner et al. 2003) in adulthood compared to controls. Thus, exposure to androgen excess early in gestation can result in changes to postnatal growth and ultimately adult body composition in rhesus monkeys. These postnatal effects mirror the phenotype in offspring of women with PCOS. It is worth noting that the primate liver and placenta have an incredible capacity to inactivate or aromatize androgens and conjugate estrogenic products compared to other species, and prenatal estrogens can influence fetal programming altering physical growth (e.g., Linn et al. 1988). Therefore, it could be argued that these effects on growth are due to estrogenic, not androgenic, action. However, testosterone treatment during a rhesus pregnancy results in a significant increase of circulating androgens but not estrogens in maternal and fetal blood (Abbott et al. 2008). This suggests that many of the symptoms of abnormal growth and obesity in adulthood are attributable to excess androgenic actions in the early intrauterine environment.

5.4.3 Prenatal Androgens and Growth: Metabolic Dysfunction and Other Potential Mechanisms

While several symptoms associated with PCOS—heavier maternal body weight and a higher occurrence of gestational diabetes (Sir-Petermann et al. 2002; Ehrmann et al. 2006; Vanky et al. 2010)—would be expected to increase the birth weight of

newborns, fetal growth is unaltered or even restricted, as evident by low birth weight in newborn with mothers with PCOS (Sir-Petermann et al. 2005). Women with PCOS and their offspring also incur metabolic dysfunction such as hyperinsulinemia and insulin resistance more often (Dunaif et al. 1987), which increases the risk of type 2 diabetes (DeFronzo 1992). As obesity, upper-body adiposity, and muscle mass can contribute to insulin resistance (Yki-Järvinen and Koivisto 1983; Bogardus et al. 1985; Caro et al. 1989; Wagenknecht et al. 2003) and women with PCOS have increased rates of obesity (Goldzieher and Green 1962; Dunaif 1992) and adiposity (Kissebah and Peiris 1989), these parameters should be normalized to understand the effects of hyperandrogenism on insulin resistance. When body composition (via a precise method such as hydrostatic weighing) and waist to hip girth ratios are similar between women with and without hyperandrogenism, there is a significant decrease in insulin-mediated glucose disposal in women with PCOS (Dunaif et al. 1992; Morales et al. 1996). This indicates that insulin resistance may be a function of hyperandrogenism rather than other confounding parameters. However, it is important to note that the onset of hyperinsulinemia in daughters of women with PCOS often precedes androgen excess, and therefore, metabolic dysregulation may actually promote the ontogeny of hyperandrogenism rather than the reciprocal relationship (Sir-Petermann et al. 2009).

Studies in prenatally androgenized female rhesus monkeys provide insight into the consequences of prenatal androgen excess on normal fetal growth and metabolism and mechanisms that can cause reprogramming of these affected tissues throughout the primate lifespan. Androgenized female rhesus monkeys early in gestation exhibit impaired pancreatic β-cell function, abnormal insulin secretion and action, hyperlipidemia, and increased abdominal fat (Abbott et al. 1998, 2005; Eisner et al. 2000, 2003). The primate pancreas expresses androgen receptors (Winborn et al. 1987), so early gestational exposure to androgen excess that coincides with pancreatic organogenesis may alter pancreatic development leading to prolonged pancreatic β-cell dysfunction (Hoar and Monie 1981). In addition, prolonged prenatal androgen exposure may be deleterious to normal placental function (de Vries et al. 1998), since placental androgen receptors are responsive to androgen action and these actions mediate changes to factors such as fibroblast growth factor 2 and folic acid that regulate fetal and placental growth (Cloke et al. 2008; Sivakumaran et al. 2010; Uzelac et al. 2010). These insults to fetal metabolism could lead to malnutrition in the fetus, independent of maternal diet and nutrition, and subsequent fetal growth restriction and adult metabolic syndrome. Although male rhesus monkeys exposed to testosterone excess during gestation do not demonstrate altered androgen production in adulthood, there is a substantial decline in insulin sensitivity and impaired pancreatic β-cell function (Bruns et al. 2004). This further supports the notion that prenatal androgens can alter programming of key metabolic tissues.

As exposure to elevated or excessive androgen levels during gestation can stifle metabolic function and alter prenatal and postnatal growth and development, it is interesting that maternal androgen levels increase during the onset of pregnancies (see Sect. 5.2.2). While these androgenic effects seem harmful to offspring

development, maternal androgen levels may reflect a fundamental trade-off in metabolic effort by pregnant females. As described in life history theory (Stearns 1992; Kaplan and Gangestad 2005), there is a trade-off for a fully developed adult to allocate metabolic efforts among one's own maintenance and reproductive efforts. Thus, changes in maternal androgens may be one mechanism through which pregnant females regulate the amount of energetic resources that would be allotted to developing fetuses. Interestingly, there are examples in primate species in which androgen production varies as a function of social instability (e.g., Batty et al. 1986; Ross et al. 2004), periods that may pose increased metabolic challenges via active territoriality and aggression. Therefore, the surge in androgens during early gestation may function to alter the metabolic demands of developing fetuses and reflect an early conflict of mothers to metabolically invest in fetal development.

5.5 Prenatal Androgens and Juvenile Behavior and Beyond

Like other mammals, sexual differentiation in the primate brain is influenced by gonadal hormones, particularly androgens, during fetal development (Goy and McEwen 1980; Ehrhardt and Meyer-Bahlburg 1981; McEwen 1981; Pardridge et al. 1982; Bao and Swaab 2010). Normal variation in or manipulation of prenatal androgen can alter the development of sexually dimorphic brain regions and sex-typical behavior that manifest throughout the lifetime in multiple primate species (Goy and McEwen 1980; Hines 2004; Cohen-Bendahan et al. 2005). Many of the behaviors influenced by prenatal androgens begin to appear—or are at least more discernable—during the juvenile/adolescent period [great apes (Watts and Pusey 2002), humans (Power 2000), callitrichines (Yamamoto 1993)]. In primates, juvenility is extended relative to other mammals, making it a period of critical interest (Pereira and Fairbanks 2002).

Some recent research focuses on normative variation in gestational androgens and childhood behavior in humans, with mixed results. These studies have evaluated gender-related play behavior and habits of boys and girls of toddler, preschool, or elementary age as a function of testosterone exposure in early midgestation. Some of these studies use the Pre-School Activities Inventory—a parent-reported assessment of sex-typical behavior in children—to measure sex-typical play behavior. From these studies, male-typical play behavior has been positively correlated with higher prenatal testosterone levels sampled from either amniotic fluid between gestational weeks 11 and 21 (Auyeung et al. 2009) or maternal serum between gestational weeks 5 and 36 (Hines et al. 2002) or masculine second to fourth digit ratio (2D:4D), the latter being indicative of high androgen exposure throughout gestation (Hönekopp and Thierfelder 2009). With substantially smaller sample sizes, Knickmeyer et al. (2005) did not find an association between prenatal testosterone in amniotic fluid collected at similar gestational ages and gender-typical play behavior in children in the same age group, nor did van de Beek et al. (2009) in 13-month-old toddlers. Thus, the effect size of this relationship may be such that measures that

are more sensitive or larger sample populations may be required. It is speculated that the period of sexual differentiation of the brain and gonads coincide (Finegan et al. 1989). As there is a significance rise in fetal androgens from gestational weeks 8 to 24 with a peak at week 19 (Tapanainen et al. 1981; Rabinovici and Jaffe 1990; Svechnikov and Söder 2008), it is during this period that androgens may have the greatest effects on brain development in androgen-sensitive regions and behavior facilitated by these regions. Hines et al. (2002) noted that there is a relatively high degree of heritability in androgen production rates; thus, mothers with high androgen levels have daughters that produce high levels of androgen. Therefore, these findings do not allow us to separate the organizational effects of prenatal androgens from the effects of circulating androgens during childhood on influencing these behaviors.

The influence of normal variation in prenatal androgens on juvenile behavior in nonhuman primates has recently been investigated in the Geoffroy's tufted-ear marmoset. In this work, normative levels of urinary testosterone, but not androstenedione, collected from mid- to late gestation in marmoset mothers are associated with social rough-and-tumble play patterns in juvenile offspring (Birnie et al. in press). Interestingly, in our study, high levels of maternal testosterone during mid- to late gestation were related to decreased rates of both receiving play and overall play (the sum of play initiations and play receptions) with siblings, but not play initiations with siblings. When we examined this same relationship with male siblings only, we found that high levels of gestational testosterone were associated with lower rates of play initiations and somewhat lower rates of overall play with male siblings, but no relationship was found for receiving play from male siblings. No relationship between maternal androgens early in gestation and offspring play behaviors was observed. Our findings stand in contrast to the results from many experimental studies conducted in rhesus monkeys, which have consistently found that experimental exposure to androgen during gestation increases rough-and-tumble play initiations with peers in genetic female rhesus macaques (see Sect. 5.5.2). However, while rhesus macaques show a sexually dimorphic pattern in juvenile rough-and-tumble play and body size (Caine and Mitchell 1979; Andrade et al. 2004), callitrichine primates exhibit few sexual dimorphism in either (Stevenson and Poole 1982; Ford 1994; Guard et al. 2002; Birnie et al. in press). In addition, maternal gestational androgens are not differentially related to the play behavior of male and female marmosets (Birnie et al. in press). Such differences in social structure and sexual dimorphism may therefore signify different developmental responses to early-life androgen exposure in primate groups.

5.5.1 Prenatal Androgen Excess on Human Juvenile Behavior and Beyond: Clinical Studies

Much of what we know about the effects of prenatal androgens on postnatal gender-typical behavior in humans comes from studying women with CAH, an autosomal disorder that causes a deficiency for 21-hydroxlase and subsequent

decreases in cortisol and increases in androgens in fetal circulation (reviewed in Merke and Bornstein 2005; White and Speiser 2000). Girls with CAH typically show a masculinized behavioral phenotype, while boys with CAH typically show few behavioral differences compared to developmentally normal boys. For instance, girls with CAH show a preference for male social play partners and male-typical toys, activities, careers, and gender identity, while boys with CAH are comparable in their toy preferences to normal boys (Berenbaum and Hines 1992; Hines and Kaufman 1994; Berenbaum 1999; Berenbaum and Bailey 2003; Pasterski et al. 2005). Interestingly, there are forms of CAH that result in variations in the severity of androgen exposure, providing a natural analog to a dose response study. Nordenström et al. (2002) noted that male-typical toy preference varied in a dose-dependent manner among girls with various types of CAH: girls with the most severe form of CAH (and therefore, the girls exposed to the highest levels of prenatal androgen) preferred male-typical toys compared to girls with more mild forms of the disorder.

A criticism of such work on early human experience is that socialization may also be shaping behavior. For instance, it is possible that girls with CAH act more like boys in their behavioral tendencies because they look more like boys and are thus treated like boys. However, in general, parents encourage gender-typical play in children both with and without CAH, but this does not attenuate the preference for male-typical toys and play activities in girls with CAH (Pasterski et al. 2005). Furthermore, 3–8-month-old infants show a sex-based difference in visual attention to gender-typical toys that mirrors patterns in toy preferences of older children (Alexander et al. 2009). If gender biases in toy preference were due solely to socialization and independent of prenatal influences, then we would expect to see no difference in visual attention paid to gender-type toys in infants who are less socialized than older children and presumably unaware of their gender identity. In addition, nonhuman primates who are not socialized in toy preferences have shown similar patterns in that male nonhuman primates prefer to play with male-type toys and females with female-type toys [vervet monkeys (*Cercopithecus aethiops sabaeus*) (Alexander and Hines 2002), rhesus macaques (Hassett et al. 2008)]. These results suggest that prenatal androgens have an organizational effect on androgen-sensitive brain regions that control these behaviors.

5.5.2 Prenatal Androgen Excess on Nonhuman Primate Juvenile Behavior and Beyond: The Rhesus Model

Much of the experimental research evaluating prenatal androgens and juvenile behavior in primates has been done in macaques. Androgens can influence the development of cortical function in rhesus monkeys. For example, the difference in direct androgen binding, testosterone aromatization, and 5α-reduction between male and female rhesus monkeys can affect the sequence of brain development (Clark et al. 1988; Clark and Goldman-Rakic 1989). Normal juvenile male

macaques initiate more play bouts (Goy and Resko 1972; Caine and Mitchell 1979), exhibit more mounting and less grooming (Goy and Resko 1972; Goy 1996), and show less interest in infants (Lovejoy and Wallen 1988) compared to normal females. Goy (1996) found that administration of TP to rhesus macaque mothers carrying female fetuses resulted in both masculinized genitalia and behavior, including increased rough-and-tumble play initiations, decreased maternal grooming, and increased mounting. Thus, androgen excess during gestation seems to promote male-typical behavior in female rhesus monkeys, potentially by altering the perception and, therefore, meaning of social cues during interactions. However, these prenatally androgenized females may have behaved differently as a result of responses from social group members to their virilized genitalia. In another study, TP treatment administered early in gestation (days 40–64) masculinized female genitalia but not juvenile play behavior (with the exception of mounting behaviors), while TP administration late in gestation (days 115–139) masculinized female play and mounting behaviors but not their genitalia or male-typical grooming behavior (Goy et al. 1988a). In follow-up research, neither blocking nor enhancing prenatal androgens at different time points throughout fetal development affected interest in infants in male and female juveniles (Gibber and Goy 1985; Kasckow et al. 2003). Thus, the masculinizing actions of androgens during gestation on behavioral development seem to be behavior-dependent, occur mainly but not exclusively later in gestation, and may be independent from androgenic effects on genital morphology. Furthermore, females exposed to lower doses of testosterone during fetal development displayed similar rough-and-tumble play compared to normal females (Eaton et al. 1990). Sexually dimorphic infant vocalizations (Tomaszycki et al. 2001) and mounting behavior (Eaton et al. 1990) were masculinized by the lower testosterone dose. Therefore, while lower prenatal testosterone doses are sufficient to affect some behaviors, the threshold for testosterone to influence play behavior is apparently higher (reviewed in Wallen and Hassett 2009).

Gestational androgen exposure has been reported to be a sufficient component in promoting male-typical copulatory behavior and diminishing female-typical sexual behavior. Sexually receptive female macaques typically solicit sexual interaction from intact males, but treatment with prenatal TP decreases this behavior (Thornton and Goy 1986). Also, pseudohermaphrodite females treated with TP during gestation and given another dose of TP prior to sexual behavior testing show higher rates of mounting a female than female controls, but not as high as intact, normal males (Pomerantz et al. 1986). Females exposed to lower doses of androgen prenatally have less masculinized genitalia but still mount females significantly more than normal females (Eaton et al. 1990). Treatment with TP or DHT proprionate during gestation also decreases attractivity and defeminizes female sexual behavior in rhesus monkeys by decreasing sexual receptivity in the presence of an intact male compared to control females (Pomerantz et al. 1985). Thus, it is likely that exposure to androgens during the prenatal and early postnatal periods accompanied by a surge in androgens during puberty is necessary to produce a masculinized sexual behavior repertoire in adulthood (Wallen 2005).

5.6 Conclusion

The organizational hypothesis proposes that nongenomic, intrauterine environmental factors such as fetal androgen can affect prenatal programming (Phoenix et al. 1959). Androgen within the intrauterine environment from maternal and exogenous sources can also cause long-term changes in multiple developmental trajectories of the fetus that can be adaptive or detrimental to postnatal survival. Thus, the plasticity of the developing fetus toward androgens and other physiological cues from the mother and environment may underlie the development of diseases, a premise postulated by the Barker hypothesis (Barker 1998). In the current chapter, we discussed a number of prenatal and postnatal developmental outcomes associated with exposure to normal variations and excessive concentrations of androgens during prenatal life in human and nonhuman primates (see Fig. 5.2). It seems that the timing of androgen exposure during gestation and the sex of the fetus are two major factors that contribute to the concentration of androgens in the prenatal environment and the ultimate outcomes. The timing of androgen exposure during gestation may influence what tissues are affected due to the variation of critical periods of develop-

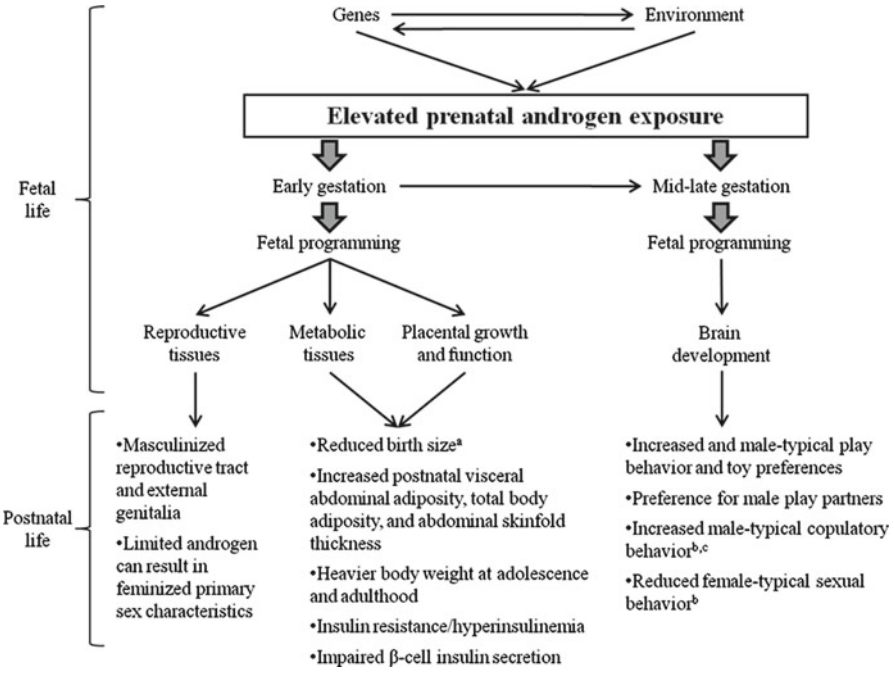

Fig. 5.2 Prenatal androgen exposure and subsequent fetal programming and postnatal outcomes. (**a**) Intrauterine growth restrictions are measureable in marmoset monkeys and a few but not all human populations and not in androgenized rhesus models. (**b**)This has only been noted in nonhuman primate species. (**c**) Androgen excess during early gestation can also promote male-typical mounting behaviors in female rhesus (Goy et al. 1988a)

ment and sensitivity for different tissue type. The sex of the offspring may dictate the influence of androgens via the sexual differences in the androgen system including androgen binding/levels, receptor distribution, and the activity of enzymes such as aromatase. The effects of normal and abnormal exposure to prenatal androgen are not limited to the sexual differentiation of the system in primates and warrant further investigation.

There are still a number of avenues that must be explored to better understand the proximate and ultimate functions of prenatal androgens on fetal programming. Such issues include specific timing and mechanisms through which prenatal androgens affect the growth and development of different tissue that are influenced by metabolic functions and prenatal testosterones, including the brain. In addition, since a number of developmental parameters are altered by prenatal testosterone such as growth, secondary sexual features, and behavior, it would be worth evaluating any mechanisms that would be developed by the offspring to compensate for insults that they would experience in their physical and social environment. This is particularly interesting as some androgenic effects seem harmful to offspring development, such as growth restrictions and metabolic dysfunction. By contrast, prenatal androgen exposure regulates normal sexual development and can promote behaviors that improve social rank, reproductive success, and allocation of territory and resources, such as social play, territoriality, and sexual behavior and receptivity. How these various altered characteristics interact to ultimately affect the life and reproductive success of an individual is a topic for further speculation and research. Not surprisingly, much more is known about physical and neuronal development in nonprimate species; however, there are ongoing research projects that focus on addressing the functions of different neurotransmitters and neural pathways associated with sexual differentiation in some nonhuman primate species. Despite the limitations in the current knowledge and technical logistics, nonhuman primates have and will continue to serve as paramount models to translate basic research findings into an understanding of normal and atypical development in humans.

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