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Environmental and social influences on neuroendocrine puberty and behavior in macaques and other nonhuman primates

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Abstract

Puberty is the developmental period when the hypothalamic-pituitary-gonadal (HPG) axis is activated, following a juvenile quiescent period, and reproductive capacity matures. Although pubertal events occur in a consistent sequence, there is considerable variation between individuals in the onset and timing of pubertal events, with puberty onset occurring earlier in girls than in boys. Evidence in humans demonstrates that social and environmental context influences the timing of puberty onset and may account for some of the observed variation. This review analyzes the nonhuman primate literature, focusing primarily on rhesus macaques (Macaca mulatta), to examine the social and environmental influences on puberty onset, how these factors influence puberty in males and females, and to review the relationship between puberty onset of adult neuroendocrine function and sexual behavior. Social and environmental factors influence the timing of puberty onset and pubertal events in nonhuman primates, as in humans, and the influences of these factors differ for males and females. In nonhuman primates, gonadal hormones are not required for sexual behavior, but modulate the frequency of occurrence of behavior, with social context influencing the relationship between gonadal hormones and sexual behavior. Thus, the onset of sexual behavior is independent of neuroendocrine changes at puberty; however, there are distinct behavioral changes that occur at puberty, which are modulated by social context. Puberty is possibly the developmental period when hormonal modulation of sexual behavior is organized, and thus, when social context interacts with hormonal state to strongly influence the expression of sexual behavior.

Keywords

Puberty onset; Social influence; Environmental influence; Sex differences; Nonhuman primate; Sexual behavior
absence, or the presence of a stepfather, indicating that environmental influences may account for some of the variation in puberty onset (Graber et al., 1995; Moffit et al., 1992; Rowe, 2000; Tither & Ellis, 2008; Wierson et al., 1993). There are sex differences in the influence that environmental factors have on the timing of puberty; for example, body weight and stepfather presence influenced the timing of puberty onset in girls, but had no effect in boys, and parental divorce resulted in earlier puberty in girls, whereas it delayed puberty in boys (Belsky et al., 2007; Bogaert, 2005; Graber et al., 1995; Rowe, 2000; Semiz et al., 2009; Wierson et al., 1993). This review discusses environmental factors that alter the timing of puberty and produce variation in the timing and tempo of pubertal change as well as sex differences in the effect of these factors in nonhuman primates. In order to provide a better understanding of the sex difference(s) in environmental regulation of puberty timing, the effects of environmental factors on puberty onset and reproductive maturation are described separately for males and females in this review. Environmental factors likely alter the timing of puberty by influencing the neurobiological factors regulating puberty onset and pace, which reflect alterations in gonadotropin-releasing hormone (GnRH) release; however, the neurobiological factors regulating GnRH release itself are an active area of investigation and discussion of these factors is beyond the scope of this review (see Plant, 2001 for a review).

**Puberty Onset**

Puberty is characterized by increased activation of the hypothalamic-pituitary-gonadal (HPG) axis as a result of increased gonadotropin-releasing hormone (GnRH) release (Suter et al., 1998; Watanabe & Terasawa, 1989). In primates, GnRH released from the arcuate nucleus of the medial basal hypothalamus acts on the anterior pituitary, resulting in the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH then act on the gonad, resulting in the synthesis and release of inhibin as well as steroid hormones such as estradiol (E2), progesterone, and testosterone (T) (Grumbach & Styne, 2003; Knobil, 1974, 1980). Inhibin released from the gonad acts on the anterior pituitary via a negative feedback loop inhibiting FSH, but not LH, release in both males and females (Figure 1; Molskness et al., 1996; Ramaswamy et al., 2000; Ramaswamy & Plant, 2001). In males, T release regulates GnRH release through a negative feedback loop (Figure 1a; Plant & Dubey, 1984). In contrast to males, in females, E2 negative and positive feedback occurs primarily at the level of the anterior pituitary and secondarily at the level of the hypothalamus (Figure 1b; Chongthammakun & Terasawa, 1993; Nakai et al., 1978; Plant et al., 1978; Xia et al., 1992). In adult female rhesus macaques, lesions of the arcuate nucleus decreased LH resulting in a cessation of ovulatory cycles, which were restored with administration of pulsatile GnRH (Plant et al., 1978). The arcuate nucleus, while required for pulsatile GnRH release, is not required for E2 feedback of the HPG axis as consistent pulsatile GnRH was sufficient to maintain ovarian cycles. No matter the site of feedback, pulsatile GnRH is required for normal ovulatory cycles and testicular function (Plant & Dubey, 1984; Plant et al., 1978).

In women and female rhesus macaques, there is an increase in progesterone concurrent with the LH surge, just prior to ovulation (Hoff et al., 1983; Knobil, 1974), but it is not clear what, if any, positive feedback effect progesterone has on the LH surge in gonad-intact females. Progesterone induces an LH surge following a small dose of E2 in ovariectomized female rhesus monkeys by increasing the frequency and amplitude of GnRH release (Terasawa et al., 1980, 1987). However, large doses of estradiol alone are also sufficient for positive feedback to occur (Terasawa et al., 1980, 1987). Thus, progesterone may facilitate positive feedback and the LH surge in females, though it does not appear to be required.
Puberty onset is marked by increased GnRH release from the hypothalamus, whereas the juvenile quiescent period of HPG activity results from inhibition of GnRH release rather than inhibition of pituitary or gonadal function (Knobil, 1980; Plant et al., 1989; Wildt et al., 1980). Administration of a glutamate agonist, N-Methyl-DL-aspartic acid (NMDA), stimulates GnRH release (Mahachoklertwattana et al., 1994). In prepubertal male rhesus macaques (15–16 months of age), intravenous administration of NMDA every 3 hours resulted in increases in LH, FSH, T, and testicular volume after sixteen weeks of treatment not observed in age-matched control males (Gay & Plant, 1987, 1988; Plant et al., 1989). Thus, GnRH was capable of being released and the pituitary was functional and able to respond to the GnRH released by NMDA treatment. Furthermore, the testes were capable of responding to gonadotropin stimulation, even though they were inactive in the untreated monkeys. When NMDA treatment was discontinued, LH, FSH, and T levels decreased to those observed prior to NMDA treatment, indicating the hormonal increases directly resulted from the GnRH released by NMDA administration (Plant et al., 1989). That the active hormone was GnRH is demonstrated by the finding that a GnRH antagonist blocked the effectiveness of NMDA treatment in increasing LH and FSH (Plant et al., 1989). Thus, the inactivity of the HPG axis during the juvenile period in males likely reflects an inhibition of GnRH release and removal of this inhibition produces an increase in GnRH release, marking the onset of puberty in males.

Similar mechanisms appear to operate in females, even though full female reproduction requires both negative and positive feedback in contrast to the need for only negative feedback in males (Chongthammakun & Terasawa, 1993; Plant & Dubey, 1984; Plant et al., 1978). Lesions of the arcuate nucleus in prepubertal females (11–15 months of age) combined with pulsatile GnRH treatment resulted in increased LH, smaller increases in FSH, and, after 2–4 LH surges luteal levels of progesterone, indicating ovulation had occurred (Knobil, 1980; Wildt et al., 1980). Thus, as in males, the juvenile female pituitary and gonad are developed and capable of responding to GnRH release well before puberty onset is typically observed, indicating that GnRH release is the factor regulating puberty onset. Lastly, levels of the inhibitory neurotransmitter, γ-Aminobutyric acid (GABA), are inversely related to GnRH levels prior to puberty and decline prior to GnRH release (Mitsushima et al., 1994). Bicuculline treatment blocks GABA_A receptor activation and results in increased GnRH release, and chronic treatment results in menarche and first ovulation occurring about one year earlier than control females (Keen et al., 1999; Mitsushima et al., 1994). Blocking GABA inhibition results in earlier increases in GnRH release and earlier puberty onset, indicating that in females, as in males, there is an inhibition of GnRH release that is removed, resulting in the puberty-initiating increase in GnRH.

In both juvenile males and females, there is an inhibition of GnRH release that is removed prior to the onset of puberty (Gay & Plant, 1987, 1988; Keen et al., 1999; Mitsushima et al., 1994; Plant et al., 1989). Although mechanisms regulating inhibition of GnRH release may differ between males and females (as reviewed by Plant, 2001), the influence of the peptide kisspeptin on GnRH release is one mechanism that appears to be similar in males and females. Increases in kisspeptin release occur prior to the pubertal increase in GnRH release and exogenous kisspeptin administration results in GnRH release in both males and females, which suggests that changes in kisspeptin release influence puberty onset in both males and females (Guerriero et al., 2012; Keen et al., 2008; Shahab et al., 2005). The mechanisms regulating juvenile GnRH inhibition are currently under investigation and a complete discussion of all of these mechanisms is beyond the scope of this review (see Plant, 2001 for a review).
In females, initiation of puberty appears to involve both the removal of inhibition of GnRH release and desensitization of E2 negative feedback (Keen et al., 1999; Mitsushima et al., 1994; Wilson et al., 1986). Prepubertal, socially housed females ovariectomized at 11 months of age did not show increased LH in response to ovariectomy until 24 months of age, indicating the removal of inhibition on GnRH secretion occurred around 24 months of age (Wilson et al., 1986). However, exposing females ovariectomized at 11 months of age to chronic E2 that produced 40–50 pg/ml blood E2 levels delayed post-ovariectomy increases in LH until approximately 30 months of age. This delayed increase in LH in the face of low-levels of E2 demonstrates decreased E2 sensitivity on negative feedback occurring approximately 5–6 months after activation of the HPG axis (Wilson et al., 1986). Thus, in gonad-intact females living in social groups, this desensitization to E2 negative feedback occurs approximately 5–6 months after the initial increase in GnRH release. It is this desensitization to E2 negative feedback that allows for increases in E2, which are required for E2 positive feedback to occur.

At the start of puberty onset, increased GnRH release results in increased levels of LH and FSH from the pituitary, ultimately resulting in increases in gonadal hormones (Grumbach & Styne, 2003; Knobil, 1974, 1980). In this review, initial increases in LH and estrogens, first appearance of sexual skin swelling (sex swelling), and menarche are all measures used to indicate puberty onset in females, whereas first ovulation, a sustained increase in luteal progesterone, is considered to be reproductive maturation. In male nonhuman primates discussed in this review, puberty onset is defined as initial increases in LH, T and other androgens, and testicular volumes. Data on environmental influences on puberty timing in males are limited to puberty onset and thus, it is not known at this time how environmental factors alter the timing of reproductive maturation in male nonhuman primates.

This sequence of pubertal events and its timing are influenced by environmental factors. In outdoor-housed rhesus macaque females, menarche typically occurs around 2.5 years of age, with first ovulation occurring at either 2.5 or 3.5 years of age (Wilson et al., 1984; Wilson et al., 1986; Zehr et al., 2005). By contrast, females living indoors experience menarche and first ovulation approximately four to six months earlier than do females living outdoors (Wilson et al., 1988). Outdoor-housed rhesus macaque males typically show increased T levels and testicular volumes, indicating puberty onset, at 3.5 years of age (Herman et al., 2006). Thus, in rhesus macaques as in humans, females enter puberty earlier than do males, with the age at puberty onset varying between individuals, and environmental context accounts for at least some of the variation in puberty timing. Because GnRH is required for gonadal function in both males and females and GnRH release is the rate-limiting factor in puberty onset (Keen et al., 1999; Mitsushima et al., 1994; Plant & Dubey, 1984; Plant et al., 1978; Plant et al., 1989), environmental factors that influence the timing of puberty onset likely alter puberty timing by altering GnRH release. This review discusses the environmental factors that regulate puberty timing, the social and hormonal factors that explain some of the variation in puberty timing between individuals, as well as changes in sexual behavior that occur around puberty. The vast majority of this review focuses on captive rhesus macaques (Macaca mulatta) because most of the research on puberty has been done in this species; however, wherever there is evidence from other nonhuman primate species that is included.

Seasonal Influences on Puberty Onset

Males

Species that have distinct breeding seasons in adulthood also have seasonal patterns of puberty onset. For example, outdoor-housed rhesus macaque males at the latitude of Atlanta, GA showed increases in testicular volume and T levels beginning in September and showed
decreases in testicular volumes and T levels in January, which continued to decline with testicular volumes becoming the smallest in April, at the end of the breeding season (Herman et al., 2006). During their first pubertal breeding season, rhesus macaque males showed increased levels of T, but lower levels than in fully adult males. This increase occurs at the same annual time as does the onset of breeding conditions in fully adult males. By a male’s second pubertal breeding season, their T levels during the breeding season were similar to those observed in adult males (Bercovitch, 1993; Herman, et al., 2006).

Day length, rather than temperature, appears to regulate seasonal patterns of T and testicular volume in rhesus macaques. Under constant temperature conditions, testicular size increased during short days (8 hr daylight) and decreased in size during long days (16 hr daylight) in individually-housed prepubertal and pubertal males (Chik et al., 1992). T levels paralleled changes in testicular volume, with T increasing along with testicular size during short days and decreasing with testicular size during long days. The effect of day length did not alter testicular size and T levels until animals entered puberty, when testicular diameters were greater than 10mm (Chik et al., 1992), which suggests that day length alters the activity of the HPG axis and the increased release of GnRH beginning at the time of puberty onset.

In species that do not show distinct breeding seasons such as wild savanna baboons (Papio cynocephalus), testicular size increases, determined by monthly visual assessment, were not seasonal, occurring in all seasons (Alberts & Altmann, 1995). Thus, male puberty onset is influenced by seasonal factors such as day length, but only in species that have a distinct breeding season in adulthood.

Females

Unlike testicular function in males, menarche in outdoor-housed female rhesus macaques was not restricted to a particular season, occurring at approximately 2.5 years of age, regardless of time of year (Wilson et al., 1986; Wilson et al., 2013). Females born during the spring or summer and females born in the fall or winter all showed menarche around 2.5 years of age (Wilson et al., 1984), but during different seasons. Thus, age at menarche appears to be more tightly linked to chronological age rather than birth season.

In contrast to menarche, first ovulation in spring-born, outdoor-housed female rhesus macaques was restricted to the fall months when females were either 2.5 or 3.5 years of age (Wilson et al., 1986; Wilson et al., 2013; Wilson & Gordon, 1989a; Zehr et al., 2005). Ovulation was not observed in the nonbreeding season (February or March to September) (Wilson et al., 1986; Wilson et al. 2013). Similarly to spring-born females, females born in the fall or winter first ovulated in the fall/winter months and were more likely to reach first ovulation around 3 years of age as opposed to 4 years of age (Wilson & Gordon, 1989a). First birth was limited to the spring and summer months regardless of which season females were born, further demonstrating that first ovulation was restricted to the fall and winter months regardless of chronological age (Wilson et al., 1984). Thus unlike menarche, first ovulation appears to be strongly influenced by season. GnRH release itself is presumably influenced by these seasonal factors as ovarietomized females, whose basal LH is elevated by the removal of E2 negative feedback, also showed decreased LH during the spring and summer (Wilson et al., 1986). This seasonal pattern in LH release is thought likely to reflect a seasonal pattern in GnRH release, which restricts the timing of first ovulation to the fall and winter months. The mechanism controlling seasonal changes in GnRH release is not known, but possibly reflects day length.

As in males, seasonal changes in female HPG function are correlated with changes in day length. Investigations of the internal signal that transduces changes in day length have focused on melatonin, with some evidence suggesting that melatonin could be that signal. In
outdoor-housed female rhesus macaques, daily melatonin injections that mimic short day (~10hr daylight) melatonin patterns were initiated at 22 months of age, which was the start of the nonbreeding season (long days), continued for about 11 months, and resulted in first sexual swelling and menarche five to six months earlier than in control animals (Wilson & Gordon, 1989b). These changes in the melatonin-treated animals occurred during the summer (nonbreeding season), whereas first sexual swelling and menarche occurred during the fall (breeding season) in control animals. Thus, melatonin patterns typically seen during shorter days may influence the timing of puberty onset, but specific melatonin patterns are not required for puberty onset as puberty onset in outdoor-housed female rhesus macaques is not limited to a specific season (Wilson et al., 1984; Wilson et al., 1986; Wilson et al., 2013; Wilson & Gordon, 1989b). First ovulation in all but one melatonin-treated animal occurred at approximately 2.5 years of age, whereas first ovulation occurred at 3.5 years of age in control females, with first ovulation occurring during the breeding season in all females (Wilson & Gordon, 1989b). Thus, exposure to levels of melatonin typically seen during shorter days resulted in earlier menarche and uniformly earlier first ovulation, but the influence of season on first ovulation in melatonin- treated females is not clear. There appears to be a minimum amount of time needed between the initial increase in LH and first ovulation as desensitization to E2 negative feedback occurs approximately 6 months after the initial increases in LH (Wilson et al., 1986). In melatonin-treated females, first ovulation coincided with the breeding season and occurred approximately 5–6 months after first sexual swelling and menarche (Wilson & Gordon, 1989b), which typically occurs within 1 month of the initial LH increase (Wilson et al., 1986, 1988). Therefore, it is not clear whether the timing of first ovulation in melatonin-treated females was influenced by seasonal factors independent of melatonin levels or if the timing of first ovulation would have occurred about six months after initial increases in LH in these females, regardless of season.

Menarche, first ovulation, and increased serum LH occurred significantly earlier in indoor-housed animals living in small groups exposed to a 12/12 hr light-dark cycle and a consistent temperature in comparison to outdoor-housed animals living in large social groups (Wilson et al., 1988). Living outdoors and exposure to seasonal changes delayed puberty onset and reproductive maturation in females. However, body size (17–26 months of age), skeletal maturation (27–42 months of age), and growth hormone levels (18–36 months of age) were significantly greater in indoor-housed animals in comparison to outdoor-housed animals (Wilson et al., 1988). Thus, exposure to seasonal changes may influence the timing of puberty onset and reproductive maturation by delaying physical growth and development. Regardless of whether females lived indoors or outdoors, first ovulation was restricted to the fall/winter months in all females (Wilson et al., 1988). The mechanism for limiting first ovulation to the fall under such different environmental conditions is not known. As with the melatonin-treated females, it is possible that first ovulation in indoor-housed animals is not influenced by season per se, but rather because first ovulation occurs approximately 6 months after initial increases in LH (Wilson et al., 1988), first ovulation happens to coincide with the breeding season. Although there appears to be a clear seasonal influence on the timing of first ovulation in outdoor-housed female rhesus macaques, the exact mechanism(s) regulating this seasonal pattern is unknown.

In wild female savanna baboons, which do not exhibit seasonal changes in gonadal function in adulthood, initial sexual swelling was not restricted to a particular season and was observed throughout the year (Bercovitch & Strum, 1993). Menarche occurred approximately 56 days after first sexual swelling, with age at initial sexual swelling and age at menarche being strongly correlated. Thus, menarche was likely not restricted to a particular season (Bercovitch & Strum, 1993). It is not known whether there are seasonal influences on first ovulation in savanna baboons, but considering adult ovarian function is
not restricted to a particular season, it seems unlikely that first ovulation would be restricted to a particular season.

**Body Weight and Puberty Onset**

It has been hypothesized that there is a critical body weight necessary for puberty onset (Frisch, 1972; Frisch & McArthur, 1974). Body weight is thought to influence the timing of puberty onset via leptin, a hormone produced by the *obese/lep* gene that signals satiety and influences fat storage (Pelleymunter et al., 1995). Mice lacking the *obese/lep* gene are infertile and leptin administration to these females restores reproductive function indicating leptin can influence activity of the HPG axis (Chehab et al., 1996). Leptin administration to normal female mice accelerates the timing of puberty onset (Ahima et al., 1997), likely as a result of altered GnRH release. Leptin can increase the release of GnRH from the hypothalamus, invoke the release of LH and FSH, and act directly on the gonads (Lebrethon et al., 2000; Yu et al., 1997). Leptin was strongly correlated with body fat in girls, with increased body fat and greater leptin levels related to earlier menarche (Matkovic et al., 1997). These results support the importance of body weight on puberty onset and suggest this effect may occur via an increase in leptin. Although leptin levels were positively related to body fat in both girls and boys at all stages, leptin increase occurred before LH and FSH peaks in girls, but was unrelated to LH and FSH levels in boys as LH and FSH continued to rise while leptin decreased (Rutters et al., 2009). The increase in T in boys throughout development appears to negatively affect leptin release (Horlick et al., 2000). Thus in humans, it appears the effect of leptin on puberty onset is limited to girls. Research in nonhuman primates allows for the direct examination of the effects of body weight and leptin on puberty onset and how these effects may differ between males and females.

**Males**

There is some evidence from rhesus macaques in support of body weight influencing puberty onset. For example, male rhesus macaques starting puberty at 3.5 years of age weighed significantly more at the start of that breeding season than males that reached puberty one year later, at 4.5 years of age (Mann et al., 1998). In addition, body weight and testicular volume were significantly correlated at 3.5 years of age (Bercovitch, 1993; Herman et al., 2006). Although these data support a relationship between body weight and puberty onset in males, T levels were not related to either body weight or testicular weight during the first two breeding seasons (Bercovitch, 1993). Thus, increased body weight is only related to puberty onset and is not related to the degree of HPG activation during puberty. The mechanism by which body weight may influence puberty onset is not clear as leptin does not appear to directly influence male puberty onset.

In juvenile male rhesus macaques living outdoors in large, social groups, leptin levels peaked at 12 and 22 months of age, well before puberty onset (Mann et al., 2000, 2002). Despite these changes in leptin, these peaks in leptin in juvenile males do not appear to influence puberty onset. Chronic leptin infusion did not alter LH levels in indoor-housed castrated prepubertal males, 16–20 months of age, which suggests that GnRH release was not altered by increased leptin levels (Barker-Gibb et al., 2002). Intravenous GnRH administration increased LH levels comparably during both leptin or vehicle infusion, further supporting that exogenous leptin does not alter GnRH release or alter the timing of puberty onset (Barker-Gibb et al., 2002). Bimonthly endogenous leptin levels did not change during the year prior to puberty onset (26–38 months of age) or during puberty (39–50 months of age), and leptin levels remained constant even though increases in LH, T and testicular volume occurred between approximately 40–46 months of age in socially-housed male rhesus macaques living outdoors (Mann et al., 2000, 2002). Thus, there are no changes in endogenous leptin levels that seem to coincide with activation of the HPG axis and
puberty onset. Although there were no observed changes in leptin levels prior to puberty, these data do not indicate whether differences in leptin levels between individuals account for some of the variation in the timing of puberty onset. In gonad-intact rhesus monkeys, leptin levels from 8–24 months and 26–50 months of age did not differ between males that reached puberty at 3.5 years of age and males that exhibited delayed puberty at 4.5 years of age, which suggests that variation in puberty timing is not influenced by leptin (Mann et al., 2002). Despite the relationship between body weight and puberty onset, the data indicate that leptin does not influence puberty onset in male rhesus macaques as leptin levels do not change around the time of puberty and differences in puberty timing are not the result of differences in leptin levels. Therefore, it is not clear how body weight may influence puberty onset in male rhesus macaques or if this relationship is simply a consequence of another factor influencing both body weight and puberty onset.

**Females**

A review of fourteen studies (2–33 females per study) found a decline in age at menarche over a fifty year period (1920s–1970s) in female rhesus macaques (Wilen & Naftolin, 1976). Consistent with this finding, Terasawa and colleagues (2012) found a decline in age at menarche from the 1970s–2000s in females at the Wisconsin National Primate Research Center (N=23), demonstrating a secular trend for earlier menarche. Despite the shift in age at menarche over time in female rhesus macaques, average body weight at menarche did not differ between time periods, supporting the hypothesis that a critical body weight is required for puberty onset (Wilen & Naftolin, 1976). A review of archival data in rhesus monkeys revealed significantly faster increases in body weight in more recent years, 2003–2005, in comparison to time periods between 1988–1990 and 1973–1975 and this significantly faster growth rate was related to earlier age at menarche (Terasawa et al., 2012). Secular trends indicate that puberty onset is occurring earlier as a result of earlier weight gain, but these trends do not provide information about how body weight relates to the timing of puberty onset on an individual level and whether body weight accounts for any variation in the timing of puberty onset between individuals. Further examination of individual archival data revealed that animals reaching menarche or first sexual swelling earlier weighed significantly less at puberty than did animals who reached puberty later. Thus, the hypothesis that a critical body weight is required for puberty onset is only supported at a population level and is not supported when examining individual data (Wilen & Naftolin, 1976). Although a critical body weight is not required for puberty onset, this evidence demonstrates that body weight as well as other growth factors may influence the timing of puberty onset.

In female rhesus macaques living outdoors, age at menarche, a mean age of 2.5 years, was not related to body weight or body mass index at menarche (Zehr et al., 2005). Although body weight at menarche was not related to age at menarche, greater increases in body weight prior to menarche (from 10–16 months of age until 26 months of age) were related to earlier menarche or first sexual swelling (Wilson et al., 2013). Thus, juvenile changes in body weight, rather than absolute body weight, may influence the timing of menarche in outdoor-socially-housed female rhesus macaques. It is possible that physical growth factors other than body weight, such as body length or growth hormone levels, which may also be related to body weight, are more influential in altering the timing of menarche rather than body weight itself. For example, patterns of body weight changes did not differ between indoor-housed and outdoor-housed females, despite earlier menarche in indoor-housed animals (Wilson et al., 1988). Increases in body length (crown-rump length) occurred prior to menarche in both indoor- and outdoor-housed females, however, indoor-housed females experienced earlier increases in body length (17–26 months of age) and greater growth hormone levels (18–36 months of age) in comparison to outdoor-housed females, indicating
that skeletal growth and/or growth hormone levels may influence age at puberty onset (Wilson et al., 1988).

Despite no relationship between body weight at menarche and age at menarche, differences in body weight and body mass index were related to the timing of first ovulation. Females that experienced first ovulation during the same season when menarche occurred, at approximately 2.5 years of age, weighed more and had a higher body mass index at 24 and 30 months of age than did females who experienced first ovulation a year later, at approximately 3.5 years of age (Zehr et al., 2005). Therefore, earlier age at first ovulation may be influenced by greater body weight and body mass index at the time of menarche (Zehr et al., 2005). Females that did not ovulate until 3.5 years of age had the greatest increase in body weight and growth hormone concentrations in the summer months, when 3 years of age, between menarche and first ovulation, demonstrating that body weight gain occurred prior to first ovulation (Wilson et al., 1984). However, differences in body weight at menarche or first ovulation are not sufficient to explain this variation in age at first ovulation as Wilson and colleagues (1986) found this variation in age at first ovulation, despite no differences in body weight at or prior to 31 months of age. Changes in growth hormone levels may also account for some of the variation in age at first ovulation. Increases in growth hormone were greater at 20–25 months of age and 26–31 months of age in females that experienced first ovulation at 2.5 years of age in comparison to females that experienced first ovulation at 3.5 years of age. Increases in growth hormone levels were observed approximately one year later in females that reached first ovulation at 3.5 years of age in comparison to females that reached first ovulation at 2.5 years of age, suggesting that growth hormone levels may also be associated with the variation in puberty timing (Wilson et al., 1986). Though body weight, body mass index, and growth hormone levels may explain some of the variation in the timing of puberty onset, it is likely that multiple growth factors interact to influence the timing of puberty onset.

Based on the data showing that developmental growth factors influence the timing of puberty onset, research manipulating the developmental growth patterns by increasing the fat content of food resulted in earlier puberty onset in females (Schwartz et al., 1988; Terasawa et al., 2012). Consumption of the higher-calorie diet resulted in first sexual swelling and menarche approximately 4–6 months earlier, and more females fed the high-fat diet ovulated at 2.5 years of age (Schwartz et al., 1988; Terasawa et al., 2012). This earlier puberty onset is not easily accounted for by body weight or body mass measures. Although Terasawa and colleagues’ indoor-housed females fed the high-fat diet had greater body weight and height than did control females, Schwartz and colleagues’ outdoor-housed females fed the high-fat diet weighed significantly less than did control females at menarche. Overall rates of weight and growth increases did not differ between females fed a high-fat diet and females fed the control diet until 27–33 months of age, at which time control animals weighed more than females fed the high-fat diet (Schwartz et al., 1988). Whether the fact that Terasawa’s females were indoor-housed while Schwartz’s females were outdoor-housed altered the relationship between weight and puberty onset is not known. It is clear, however, that the effect of a high-fat diet on puberty onset is not clearly associated with increases in body weight; rather, changes in body weight may be a proxy for caloric flux, which may be what is influencing puberty onset (as reviewed by Schneider et al., 2012). Terasawa and colleagues found the high-fat diet resulted in a greater body mass index and abdominal fat measures, as well as greater leptin levels. In contrast, Schwartz and colleagues found that consumption of a high-fat diet resulted in lower body fat at the time of menarche, but this difference in body fat did not differ by treatment at the time of first ovulation. Although earlier puberty onset with a high-fat diet was found in both studies, the data clearly show that body weight or body fat are not determinative and are at best a correlate of the factors that regulate puberty onset. Something like energy availability, which
may correlate under some conditions with body weight and not under other conditions (e.g. of high energy expenditure), could be the common mechanism by which puberty onset varies.

In gonad-intact, indoor-housed female rhesus macaques, both diurnal and nocturnal leptin levels were significantly higher at the age when the nocturnal rise in LH was detected, an early sign of activation of the HPG axis, in comparison to leptin levels prior to the nocturnal rise in LH (Wilson et al., 2003). Leptin administration from 12–30 months of age hastened the nocturnal rise in LH, sexual swelling, age at menarche, and age when E2 levels became detectable, but this was not accompanied by differences in body weight between leptin-treated and control females. Although leptin administration resulted in earlier puberty onset, it did not influence the timing of first ovulation in indoor-housed females (Wilson et al., 2003). Serum leptin levels were significantly lower in outdoor-housed, low-ranking females from 24–30 months of age in comparison to high-ranking females (Wilson & Kinkead, 2008). This effect of decreased leptin levels in low-ranking females was due to lower leptin levels from 24–27 months of age in subordinate females with a specific genetic polymorphism. Low-ranking females with at least one allele coding for a short promoter region, 5HTTLPR, on the serotonin transporter gene, SLC6A4, experienced later first ovulation in comparison to all high-ranking females, regardless of the presence of this polymorphism, and low-ranking females without this polymorphism, indicating that the effects of leptin on first ovulation are likely influenced by both genetic and social factors (Wilson & Kinkead, 2008).

In summary, in males, body weight bears no clear relationship to gonadal function and leptin has no influence on the timing of puberty onset. In contrast, in females, body weight, body mass, physical body growth, and diet content can be related to the timing of puberty onset, though differences in the timing of puberty onset are not always related to these growth factors. Leptin and growth hormone concentrations are two potential mechanisms by which body growth influences the timing of puberty onset in females.

Social Rank and Puberty Onset

Males

In wild male savanna baboons living in multi-male/multi-female groups, the onset of puberty is characterized by increases in testicular size and the end of the pubertal transition to adulthood is marked by dispersal from the natal group or attaining status within the adult male hierarchy of the natal group (Alberts & Altmann, 1995). Prior to developing their own social rank, male savanna baboons inherit their natal group rank from their mothers and this is the male’s rank at the time of puberty onset. Maternal rank was strongly related to increases in testicular size as higher-ranking male savanna baboons showed earlier increases in testicular size than did lower-ranking males (Alberts & Altmann, 1995; Charpentier et al., 2008). A similar relationship between maternal rank and puberty onset was found in male mandrills (Setchell et al., 2006). Maternal social rank was similarly related to the age when males attained adult social rank (Alberts & Altmann, 1995), with males from higher maternal ranks attaining adult social rank earlier that did males from lower-ranking mothers. This suggests that maternal rank is also related to the timing of the culmination of puberty in male savanna baboons. If high social rank results in earlier testicular development as well as shortens the period of time between testicular development and age of first adult rank, then it is possible that maternal rank influences these two factors independently. However, if the length of puberty in males does not vary by maternal rank, then that would suggest that maternal rank influences age of puberty onset and the relationship between maternal rank and age at first adult rank is simply a result of earlier puberty onset in higher-ranking males. Although the influence of maternal social rank on factors occurring later in the pubertal
period is not clear, it is clear that higher maternal social rank is related to earlier testicular development in males.

As in wild savanna baboons, in male rhesus macaques living in multi-female/one-male groups, maternal social rank was related to both testosterone levels and testicular weight during the breeding season when males were approximately 3.5 years of age (Dixson & Nevison, 1997). Male offspring from higher-ranking mothers had both higher T levels and greater testicular weights (Dixson & Nevison, 1997), which is consistent with the data from savanna baboons showing high maternal rank was related to earlier testicular growth (Alberts & Altmann, 1995). T levels and testicular weight were also correlated, with higher T levels being related to greater testicular weight, which suggests testicular weight is a valid proxy for T levels in adolescent males when blood collection is not possible (Dixson & Nevison, 1997). Though maternal rank appears to influence aspects of pubertal development such as T levels and testicular weight, this study was limited to a single season of data collection and does not provide data on whether maternal rank is related to the timing of puberty onset.

Around the time of puberty onset, male rhesus macaques remaining in their natal group are integrated into the linear male hierarchy (Koford, 1963) and thus, rank within the male hierarchy may also influence the timing of puberty. In male rhesus macaques living outdoors in large, multi-male/multi-female groups, social rank within the male hierarchy was not related to maximum testicular volume or increases in serum LH and T following a dose of exogenous GnRH at 3 years of age, prior to the first pubertal breeding season (Herman et al., 2006). However, by 3.5 years of age, the age of puberty onset in male rhesus macaques, social rank was related to maximum testicular volume, mean serum T and LH levels (September-January), and responsiveness of the HPG axis (Bercovitch, 1993; Herman et al., 2006). At 3.5 years of age, higher-ranking males had larger testicular volumes, higher endogenous serum T and LH levels, and higher T and LH levels in response to an injection of GnRH, which suggests that during the first pubertal breeding season, higher-ranking males experienced greater activation of the HPG axis in comparison to lower-ranking males (Bercovitch, 1993; Herman et al., 2006).

During the nonbreeding season at 4 years of age, social rank continued to be related to testicular volume, but not to serum T and LH levels (Herman et al., 2006). Despite the lack of a relationship between social rank and T or LH levels at 4 years of age, increases in T following a GnRH injection remained related to social rank, and there was a trend for a relationship between social rank and increases in LH following exogenous GnRH. Thus, social rank is still related to an increased responsiveness of the HPG axis at 4 years of age, but this effect is not naturally observed likely due to lower levels of circulating T and LH during the nonbreeding season (Herman et al., 2006).

During the second pubertal breeding season, 4.5 years of age, social rank remained related to testicular volume, there was trend for a relationship between social rank and serum LH, but there was no relationship between social rank and serum T (Herman et al., 2006). Consistent with these findings, there was a trend for a relationship between social rank and LH response to exogenous GnRH, but there was no relationship between social rank and T response to exogenous GnRH. Thus, at 4.5 years of age, the trend for a relationship between social rank and LH levels or LH response to GnRH suggests that higher-ranking animals are producing more LH, but that this increase in LH is not resulting in increased T levels. In summary, in male rhesus macaques, social rank is related to greater responsiveness of the HPG axis at 3.5 and 4 years of age, but this effect disappears, with the exception of differences in testicular volume, by 4.5 years of age (Herman et al., 2006).
Bercovitch and colleagues (1993) found that high-social rank predicted earlier seasonal increases in T in pubertal males and lower-social rank was associated with delayed seasonal increases in T, but peak T levels during the breeding season did not vary with rank. Thus, the effects of social rank in male rhesus macaques are likely limited to the initial increases in T during the breeding season. The mechanism(s) regulating the relationship between social rank and activation of the HPG axis are not clear, but this effect of social rank is likely not mediated by greater body weight in high-ranking animals as there was no relationship between social rank and body weight, despite relationships between testicular volume and both social rank and body weight (Bercovitch, 1993; Mann et al., 1998; Herman et al., 2006).

**Females**

Female social rank was related to age at puberty onset in savanna baboons with dominant females having an earlier age at first sexual swelling and menarche (Bercovitch & Strum, 1993; Charpentier et al., 2008). Age at first sexual swelling was positively correlated with age at menarche and menarche occurred, on average, two months after first sexual swelling (Bercovitch & Strum, 1993). The interval between first sexual swelling and menarche did not differ between high and low-ranking females (Bercovitch & Strum, 1993), which suggests that the influence of social rank is limited to puberty onset and once puberty is initiated, social rank does not further predict the timing of pubertal markers. Interestingly, age at first birth did not vary by social rank, suggesting that earlier puberty onset does not result in greater reproductive success (Bercovitch & Strum, 1993). Family and group structure also appear to influence the timing of menarche in savanna baboons as earlier menarche was associated with more adult female siblings as well as fewer female group members (Charpentier et al., 2008).

In contrast to savanna baboons, in female rhesus macaques, social rank was not related to age at first sexual swelling (Wilson & Kinkead, 2008) or age at menarche when all females reached menarche around 2.5 years of age (Wilson & Kinkead, 2008; Zehr et al., 2005). The lack of association between female social rank and puberty onset may reflect the lower variation in age of menarche in rhesus macaques than in savanna baboons (Bercovitch & Strum, 1993; Wilson & Kinkead, 2008; Zehr et al., 2005). These findings need to be interpreted cautiously as higher social rank was found to be associated with earlier puberty onset when females were studied year round beginning at approximately 1 year of age and when puberty onset is defined as either menarche or first sexual swelling (Wilson et al., 2013). The exact relationship between sexual swelling and menarche in female rhesus macaques is unknown, but both are likely to reflect underlying neuroendocrine events; however, whether the events they reflect are the same for both endpoints is currently unknown. At this point it is unclear whether higher social rank predicts earlier menarche.

Age at first ovulation, however was clearly related to social rank with higher-ranking females reaching first ovulation at an earlier age (Wilson et al., 2013). When social rank (high, middle, and low) and age at first ovulation (2.5 or 3.5 years of age) were grouped for analysis, high- and middle-ranking females first ovulated at either 2.5 or 3.5 years of age, whereas all low-ranking females experienced first ovulation at 3.5 years of age (Zehr et al., 2005). These data demonstrate that in female rhesus macaques, higher social rank does not uniformly predict earlier first ovulation, but rather low-social rank delays first ovulation. Although age at first ovulation varied by social rank, there were no differences in body weight at first ovulation based on social rank (high, middle, low) indicating that differences in body weight at 2.5 years of age may explain the variation in the timing of first ovulation (Zehr et al., 2005). Females that first ovulated at 2.5 years of age had greater body weights and higher body masses at this age in comparison to the body weights and body masses at 2.5 years of age of females that ovulated a year later. In addition, low-ranking females...
weighed significantly less than high- or middle-ranking females at 2.5 years of age. It is possible that age at first ovulation is regulated primarily by body weight and that lower-ranking females may ovulate later due to lower body weights at the time of menarche (Zehr et al., 2005). However, it is equally likely that delayed puberty and lower body weight are both a consequence of low social rank and that weight per se is not the cause of the delayed puberty in lower-ranking females. Wilson and Kinkead (2008) found no social rank differences in weight gain between birth and 2.5 years of age despite finding delayed ovulation in low-ranking females, which supports the idea that the effect of social rank on first ovulation is not mediated through differences in body weight per se but rather that social rank may influence body weight and puberty onset independently.

Puberty onset, defined as menarche or first sexual swelling, was related to age at first ovulation, and the time between puberty onset and first ovulation was related to age at first ovulation, suggesting that the period of adolescent sterility between puberty onset and first ovulation was shorter in females that ovulated earlier (Wilson et al., 2013). Distinct behavioral experiences as a juvenile (10–16 months until 26 months of age) predicted age at puberty onset and age at first ovulation in female rhesus macaques, which suggests that early social experience may influence puberty timing (Wilson et al., 2013). Juvenile females that received more submissive gestures from members of the group, which would be characteristic of higher-ranking females, reached menarche or first sexual swelling earlier than females receiving less submissive gestures from the group. Less aggression received from group members, less submissive behavior directed towards others, higher rates of affiliation towards others, and greater weight gain from 10–16 months until 26 months of age, which are all consistent with behaviors observed in higher-ranking animals, predicted earlier first ovulation. Lastly, showing higher levels of submission to other group members, which is characteristic of lower-ranking females, predicted a longer duration between puberty onset and first ovulation, indicating that behaviors characteristic of low-ranking females resulted in a longer duration of puberty (Wilson et al., 2013). In summary, behaviors consistent with high social rank predicted earlier puberty onset and first ovulation, whereas behaviors consistent with low social rank predicted a longer duration of puberty. Although the data demonstrate that early social experience may predict puberty timing, it is not clear whether social rank or behavioral patterns are predicting puberty timing. It is possible that distinct behavioral patterns influence an animal’s social rank and thus, behavioral patterns are influencing the differences in puberty timing. Alternatively, it is possible that social rank influences behavioral patterns and social rank is the factor influencing puberty timing.

The influence of social rank is more pronounced in new world monkeys than that seen in old world primates. In adult female common marmosets (Callithrix jacchus), a pair-bonding new world primate, dominant females suppress ovulation in subordinate females and this suppression continues as long as the subordinate female is exposed to the scent of the dominant female (Abbott et al., 1988; Barrett et al., 1990). This ovulation suppression does not likely reflect increased stress in the subordinate female, as circulating levels of cortisol are not higher in subordinate females (Abbott et al., 1981). Additional research shows ovulation suppression in adult female marmosets is not a result of decreased hypothalamic GnRH release in subordinate females as GnRH release in breeding females in the follicular phase was similar to GnRH release in subordinate females (Abbott et al., 1997). Thus, in the case of marmosets, social context appears to be influencing the pituitary and/or the ovary, rather than the hypothalamus, which suggests that in juvenile females this suppression prevents first ovulation, but not puberty onset.

Captive female common marmosets first ovulated at approximately 400 days (13 months) of age, but this coincided with removal from the natal group and introduction to a new group.
Therefore, it is not clear whether first ovulation occurred as a result of reaching a particular age or from changes in the social context (Abbott & Hearn, 1978). When remaining in the natal group, about 46% of subordinate females ovulated at least once, indicating that first ovulation can occur in the natal group, and females that do not show behavioral submission to their mothers are more likely to ovulate in the natal group (Saltzman et al., 1997, 2004; Sousa et al., 2005). Removal of the male and introducing a new male to the group significantly increased the proportion of females ovulating while in their natal group, however, when accounting for mother-daughter dominance relationships in the analysis, introducing a new male to the group did not significantly increase the number of ovulating females (Saltzman et al., 1997, 2004). Thus, the data suggest social subordination in the natal group suppresses ovarian function in that daughters that are behaviorally subordinate to their mothers are less likely to ovulate than less subordinate daughters.

In contrast to ovarian suppression, suppression of sexual behavior in captive female common marmosets in the natal group appears to be influenced by the presence of the father (Saltzman et al., 2004). Captive female marmosets in their natal group did not engage in sexual behavior, but when the father was removed and an unfamiliar male was introduced, daughters showed an increase in sexual behavior towards the new male. Both anovulatory and ovulatory females showed increased sexual behavior towards the new male, but females having ovulatory cycles during the behavioral collection period showed a greater increase in sexual behavior than did anovulatory females (Saltzman et al., 2004). Thus, suppression of ovarian function and sexual behavior appear to be regulated by different mechanisms in captive female common marmosets.

In wild common marmosets, ovarian suppression was not observed in the subordinate female, but rather it appeared that sexual behavior was influenced by social rank (Sousa et al., 2005). The dominant female mated exclusively with the breeding male, whereas subordinate females may have attempted to mate with the breeding male, but extragroup copulations were also observed (Sousa et al., 2005). These data suggest that mating attempts by subordinate females, not ovarian function in these females, are largely regulated by the dominant female in wild common marmosets. Thus, suppression or regulation of sexual behavior appears to occur in both wild and captive female common marmosets; however, ovarian suppression may be a consequence of living in captivity.

In captive female Wied’s black tufted-ear marmosets (Callithrix kuhli), urinary progesterone metabolites and LH levels were low in females less than one year of age, indicating the HPG axis was not activated (Smith et al., 1997). Spontaneous ovulation in females living in their natal group occurred at approximately 15.6 months of age, but these young females had shorter luteal phases accompanied by lower levels of progesterone metabolites in comparison to when housed alone. Young females living in their natal group also had significantly lower progesterone levels than adult breeding females and their luteal phase length also showed a tendency to be longer. Length of the luteal phase did not differ between young females that were housed alone and adult breeding females. Despite the occurrence of spontaneous ovulation in this species, remaining in the natal group appears to influence ovarian function in the luteal phase in young females (Smith et al., 1997).

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In cotton-top tamarins (Saguinus oedipus), a pair-bonding species, female offspring living with their family begin showing elevations of urinary LH and estrogens between 15 and 17 months of age, indicating activation of the HPG axis and puberty onset (Ziegler et al., 1987). However, these females did not show cyclic hormonal changes and had significantly lower levels of LH and urinary estrogen conjugates than did the dominant female, their mother. By 26–29 months of age, daughters still showed suppressed ovarian function if they remained in the natal group. Removing a daughter from her family group and housing the female in

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isolation, preventing visual and olfactory cues from others, did not result in ovulation, indicating removal from the family group is not sufficient to induce ovulation after ovarian suppression. Although urinary estrogen conjugates and LH levels increased in individually-housed females when exposed to auditory and olfactory cues from others, ovulation did not occur until the female was paired with a novel male. Thus, exposure to a novel male is required for adult ovarian function in young, ovarian-suppressed female tamarins (Ziegler et al., 1987).

Removal of a daughter from the family unit prior to puberty onset, at 9 months of age, followed by exposure to an unfamiliar male accelerated puberty onset in a female tamarin and elevated urinary LH and estrogen conjugates by 10 months of age, 5–7 months earlier than was seen in the family unit (Ziegler et al., 1987). It is not known whether introduction to the novel male at 9 months of age also resulted in earlier ovulation, as a result of the observed earlier increases in LH and estrogen conjugates, or if age at first ovulation was unaffected by this earlier activation of the HPG axis.

In species such as marmosets and tamarins that live in family groups, social context may delay first ovulation, but it is not clear how it affects the timing of puberty onset. It is difficult to determine whether puberty onset, as indicated by increased LH and estrogens, is suppressed in daughters living in a family group, accelerated in daughters removed from the family group and introduced to a novel male, or whether both acceleration and suppression of puberty onset occur in these species.

The effect of gonadal suppression due to remaining in the natal group is limited to females as captive male cotton-top tamarins living in their natal group had urinary LH, T, and dihydrotestosterone (DHT) levels that were comparable to their father, the breeding male in the group (Ginther et al., 2001, 2002). Thus, sons living in the natal group were capable of producing offspring, but mating attempts with females, either their mother or sisters, were rare and mounts were directed towards other males in the group (Ginther et al., 2001). Similar to male cotton-top tamarins, captive male golden lion tamarins (Leontopithecus rosalia) living in their natal group did not show decreased fecal androgen levels in comparison to their father (Bales et al., 2006). In addition to tamarins, captive male common marmosets showed increases in testicular volume and plasma T levels around 250 days (8 months) of age, while in the natal group, and their T levels were comparable to those observed in adult males (Abbott & Hearn, 1978). Similarly, levels of urinary androgens in white-faced marmosets (Callithrix geoffroyi) increased throughout development and by 16–24 months of age, urinary androgen levels of sons living in the natal group did not differ from their father, the breeding male in the group (Birnie et al., 2011). Thus, in tamarins and marmosets, males appear to go through puberty in the natal group without any evidence of gonadal suppression. However, male marmosets and tamarins have not been subjected to as many social manipulations as have females, so we don’t know for certain that pubertal events are not sensitive to social context in sons as they are in daughters.

Despite a lack of testicular suppression in their natal group, testicular suppression has been observed in subordinate golden lion tamarins unrelated to the dominant male (Bales et al., 2006). However, such testicular suppression was not observed if the subordinate male was related to the dominant male, suggesting that gonadal suppression can occur in some social contexts in males, but may depend on the relationship or interactions between the pair of males. Thus, both males and females show gonadal suppression but the social contexts in which this occurs may differ by sex.
Pre- and Neonatal Testosterone Levels and Puberty Onset

A sex difference in the age at puberty onset exists in humans in that girls enter puberty earlier than boys (Marshall & Tanner, 1969; 1970). This sex difference in puberty onset has also been documented in some nonhuman primate species, such as rhesus macaques (Zehr et al., 2005; Herman et al., 2006). One potential hypothesis for this sex difference is that elevated pre-and neonatal testosterone organizes the HPG axis in males such that early androgen exposure delays puberty onset in males in comparison to females. This section reviews the evidence on the influence of pre- and neonatal testosterone exposure and the relationship to puberty onset.

Males

Exposing fetal rhesus macaque males to exogenous prenatal T for 30–35 days either early (beginning between gestational days 35–40) or late (beginning between gestational days 110–115) in a 170 day gestation had no effect on testicular volumes, T levels, or LH levels in comparison to control males between 3.5 and 4.5 years of age (Herman et al., 2006). However, the dose of T used (20mg/kg testosterone cypionate per week) was not particularly large as it was insufficient to masculinize the genitals of female fetuses exposed to this androgen treatment (Herman et al., 2000). Thus, it is possible that because of the negative feedback effects of exogenous T, which would reduce endogenous androgens, that the fetal males were not actually exposed to elevated levels of androgens.

In contrast to the lack of effects of prenatal androgen treatment, males treated prenatally with flutamide, an androgen receptor blocker, early in gestation had significantly larger testicular volumes and higher T and LH levels at 3.5 years of age in comparison to control males, who did not show comparable testicular volumes and T and LH levels until 4.5 years of age (Herman et al., 2006), suggesting earlier puberty onset. Interestingly the genitals of these early flutamide-treated males were significantly more female-like and less masculinized than those of control males and these males also showed a female-typical earlier puberty onset (Herman et al., 2000; Herman et al., 2006). This finding is consistent with prenatal androgen organizing the later puberty onset in males compared to females.

In contrast to its effects when administered early in gestation, flutamide administered late in gestation did not result in higher levels of T or LH at 3.5 years of age, although testicular volumes were significantly larger than in control males (Herman et al., 2006). The source of the increased testicular volume is not known, but suggests the effects of prenatal flutamide exposure on the organization and later functioning of the HPG axis are limited to early gestation.

By 4.5 years of age, there were no longer any differences between prenatally flutamide-treated and control males (Herman et al., 2006). Thus, flutamide treatment specifically affected the timing of puberty onset and did not produce subsequent differences in HPG function. Although a number of critical experiments remain to be done, the most consistent explanation is that elevated prenatal T early in gestation delays puberty and blocking this elevation advances puberty onset in males.

In addition to the prenatal activation of the HPG axis, males show a neonatal increase in T during the first three months of life (Mann et al., 1984, 1989). Neonatal suppression of the HPG axis in males using a GnRH agonist (D-Trp⁶-N-α-Me-Leu⁷-des-Gly¹⁰-Pro⁹-NH₂-GnRH) for 112 days beginning at 10–13 days of age suppressed this neonatal T surge and resulted in blunted T levels during the pubertal period in male rhesus macaques (Mann et al., 1989, 1993). At 3 years of age, GnRH-agonist-treated males had significantly lower T levels before and in response to an intravenous injection of GnRH in comparison to control males.
though the pattern of increase in T was similar in both control and treated animals. However, serum LH levels in response to a GnRH injection did not differ by treatment (Mann et al., 1989). These data suggest that neonatal GnRH-agonist exposure, which suppressed neonatal T, resulted in decreased testicular sensitivity to LH or that the testicles were less developed and thus, produced less T in response to the increased LH levels. It is possible that differences in LH were not observed because samples were collected monthly and that more frequent sampling may indicate lower serum LH levels in GnRH-agonist-treated animals. At 3.5 years of age, GnRH-agonist-treated males had blunted T levels, lower testicular volumes, and lower sperm counts in comparison to control males, despite no apparent differences in LH levels (Mann et al., 1989, 1993). Half of the neonatally GnRH-agonist-treated animals failed to show an increase in serum T or testicular volumes at 3.5 years of age, likely a result of lower serum LH levels in these males in comparison to GnRH-agonist-treated animals that showed increases in T or testicular volumes (Mann et al., 1989, 1993). Thus, GnRH-agonist treatment resulted in delayed testicular development, which suggests that neonatal T exposure organizes the HPG axis and influences the sensitivity of the testes, which interferes with the timing of puberty onset. Differences in serum LH and T as well as testicular volumes between GnRH-agonist-treated males and control males disappeared by 5.5 years of age, indicating the delayed development resulting from suppression of neonatal T did not permanently alter the functioning of the HPG axis and treatment differences were limited to the timing of puberty onset (Mann et al., 1993). GnRH-agonist treatment did not significantly influence body weight at puberty and therefore, cannot account for the observed effects (Mann et al., 1989). Interfering with neonatal T resulted in delayed puberty onset (Mann et al., 1989, 1993), the male-typical pattern, whereas blocking prenatal T’s effects resulted in earlier puberty onset (Herman et al., 2006), the female-typical pattern. Thus, T appears to have opposing effects depending on the developmental timing of exposure to T.

GnRH agonists produce an initial large increase in FSH, LH, and T (Akhtar et al., 1983; Heber et al., 1984). Possibly, these elevated hormones were the mechanism by which puberty onset was delayed. GnRH antagonists don’t produce this initial hormonal increase. To investigate the effect of complete neonatal T suppression, rhesus macaque males were treated with vehicle, GnRH antagonist (antide), or antide + T beginning during the first two days of life and continuing through 4 months of age (Mann et al., 1998). The T treatment was designed to be a replacement dosage, but ended up producing higher T levels than in controls and prolonging the neonatal T elevation to 4 months instead of the 3 month endogenous T increase. Males first showed increases in testicular volume at 3.5 years of age, but pubertal increases in T and LH occurred at either 3.5 or 4.5 years of age, signaling puberty. Males reaching puberty at 3.5 years of age did not differ by treatment in LH levels, T levels, or testicular volumes. However, significantly fewer antide- or antide + T-treated males had puberty onset at 3.5 years of age compared to control males and this delayed puberty onset was not related to body weight or social rank (Mann et al., 1998). Thus, antide treatment, regardless of whether it was combined with T treatment, resulted in significantly more males experiencing later puberty onset, which supports neonatal organization of the timing of puberty onset that may involve GnRH release, or androgens, or other factors. However, another possibility is that antide treatment altered the development of immune function and that this in turn delayed puberty onset as both antide treatment and the combined antide plus T treatment altered responsiveness of the immune system (Mann et al., 1994, 1999).

Whether such immune system changes affect puberty timing cannot be reconciled without further research specifically investigating this question. However, both neonatal GnRH-agonist and -antagonist treatment, as well as the combined antagonist plus T treatment, resulted in more males experiencing later puberty onset and altered immune function (Mann...
et al., 1994). However, in males that reached puberty at 3.5 years of age, those treated neonatally with a GnRH antagonist did not have different T levels than did controls, whereas neonatally GnRH-agonist-treated males had blunted pubertal T levels (Mann et al., 1989, 1993, 1998). Thus, the initial hormonal elevations produced by neonatal GnRH-agonist treatment may alter the sensitivity of the pituitary and/or the gonad to hormones, thereby influencing negative feedback, or treatment may simply result in delayed testicular development. Although the mechanisms operating during the neonatal period remain unresolved, evidence strongly supports that the early neonatal period is a sensitive period for hormones or immune factors affecting the timing of puberty onset.

Females

In contrast to male rhesus monkeys, in female rhesus monkeys, manipulating prenatal androgen exposure did not alter the timing of puberty. Prenatal exposure to androgen or flutamide, an androgen receptor blocker, for 30 days either early (beginning at day 35–40) or late in gestation (beginning at day 110–115) did not influence the timing of menarche or first ovulation in female rhesus macaques (Zehr et al., 2005). Previous work in rhesus monkeys reported later menarche in females exposed to very large amounts of prenatal androgen early in gestation (Goy et al., 1988). However, the genitals of Goy’s early-androgen-treated females were almost completely masculinized and had no vaginal opening, precluding detecting menarche using vaginal swabs as was used by Zehr and colleagues (2005). In the Goy study, menstrual blood exited through androgenized-female’s penile urethra and thus, it is unlikely the light bleeding typical of menarche would be detected, but rather only the heavier bleeding typical of menstruation following ovulation would be detected. Thus, the later menarche in the Goy study may actually reflect first ovulation. Unfortunately, first ovulation was not measured in the Goy study so it unknown what the delayed menarcheal bleeding represents. It is possible that the much higher exogenous T used in Goy’s study actually delayed menarche, or equally plausible is that true menarcheal bleeding was not detected.

Further evidence of sex differences in the sensitivity of timing mechanisms come from the finding that neonatal suppression of gonadal activity in female rhesus macaques using a GnRH agonist, Lupron depot, from birth to approximately eight months of age, did not affect age at menarche, age at first ovulation, or the time interval between menarche and first ovulation (Wilson & Kinkead, 2008). Taken together these data suggest that unlike in males, in females, the HPG axis is not organized during prenatal or neonatal time periods and that hormonal manipulations at these times do not alter the timing of pubertal events. Thus, the sex difference in the timing of puberty appears to reflect androgens both pre- and post-natally lengthening the time to puberty in males above that seen in females. Why these neuroendocrine mechanisms are sensitive to prenatal or neonatal androgens in males, but not females is not known.

Pubertal Changes in Behavior

Males

Male macaques display mounting behavior typically within the first three months of life, but these mounts are not accompanied by intromissions or ejaculatory reflexes (as reviewed by Wallen, 1996, 2005). However, there is some evidence to support that full copulatory behavior occurs before endocrine puberty. Male stumptail macaques (Macaca arctoides), who mount frequently as young juveniles, began showing intromissions around 1.5 years of age, with the first observed ejaculatory reflex occurring shortly thereafter, though first seminal fluid emission did not accompany the ejaculatory reflex until approximately four years of age (Nieuwenhuijsen et al., 1988). At 1.5 years of age, behavior was not related to
body weight or maternal dominance rank. Thus, juvenile stump tail males have the capacity for full copulatory behavior prior to puberty onset, despite the endogenous suppression of the HPG axis and lack of gonadal hormones. Copulation frequency did not significantly increase until about one year prior to testicular descent and was not related to body weight, T levels, or maternal dominance rank. Thus, an increase in copulatory behavior occurred prior to puberty onset, indicating that the development of the capacity to copulate occurs independently of and is not dependent on gonadal maturation (Nieuwenhuijsen et al., 1988).

Evidence in rhesus macaques also demonstrates the capacity to copulate develops independent of endocrine and behavioral puberty (Wallen, 2001). The components of male sexual behavior (erection, mounting, and intromission) are present well before endocrine puberty. However, the frequency of occurrence of each of these components, as well as the ejaculatory reflex, increases concurrently with endocrine puberty (Wallen, 2001). Ejaculatory reflexes have rarely been reported in male rhesus macaques prior to puberty, but from studies of long-term castrated males we know that T is not required for expression of the ejaculatory reflex (Chambers and Phoenix, 1983; Phoenix et al., 1973). Although the expression of the ejaculatory reflex doesn’t require T, suppressing endogenous T reduces ejaculatory frequencies, likely due to decreased sexual motivation (Wallen et al., 1991).

Though endocrine puberty is not necessary for males to express the components of male sexual behavior, the frequency of expression and the sex copulatory behavior is directed towards changes with puberty. Male rhesus macaques routinely display the stereotyped double-foot-clasp mount used in adult copulation throughout the juvenile period, mounting male and female partners equally (Figure 2; Wallen, 2001). During the peripubertal period male mount rate more than triples, with the increase resulting from an almost 8 fold increase in mounting of female partners (Figure 2). This transition from not discriminating the sex of the mounting partner to almost exclusive mounting of females is best predicted by whether a male was observed to display an ejaculatory reflex with a female, whether or not he had shown a pubertal increase in T (Figure 3; Wallen, 2001). Although most males had increased T prior to their transition to exclusively mounting females, one male who did not have elevated T showed both the ejaculatory reflex and increased mounting of females (Wallen, 2001). Though pubertal increases in T likely increase the probability that males will become sexually involved with females, it is apparent that increased T is not obligatory for this change in male sexual behavior. Part of the function of pubertal T increases is to offset the effects of social context on male sexuality.

At 3.5 years of age, during their first pubertal breeding season, higher rates of mounting with adult and pubertal females was related to higher social rank, larger testicular volume, greater T levels, and greater LH levels in male rhesus macaques (Herman, et al., 2006). During the second pubertal breeding season, 4.5 years of age, mounting rate was still related to social rank, but was not related to T or testicular volume (Herman et al., 2006). This restriction of a relationship between LH levels, T levels, and testicular volume to mounting rate suggests that males showing greater activation of the HPG axis at 3.5 years of age are showing higher rates of mounting. By 4.5 years of age, rank related differences in T and LH no longer exist, indicating the influence of social rank on sexual behavior is not related to differences in activation of the HPG axis (Herman et al., 2006). Social rank begins exhibiting an influence on sexual behavior at puberty and continues to influence mounting rate throughout puberty. By contrast, social rank was unrelated to the rate of masturbation throughout puberty, indicating the effects of social rank on mounting rates do not reflect differences in sexual motivation (Herman et al., 2006).

One possible reason that social rank affects male sexual behavior is that females selectively avoid mating with low-ranking pubertal males and thus, only high-ranking pubertal males
had access to females. This seems likely as adult males showed higher rates of mounting and intromissions than did pubertal males (Dixson & Nevison, 1997). However, this difference does not appear to reflect differences in pubertal males’ sexual interest as they visually inspected and sniffed female genitalia at levels comparable to those seen in adult males (Dixson & Nevison, 1997). Thus, pubertal males are sexually interested in females, but most do not have the opportunity to mate with females. Females showed fewer sexual solicitations and initiated proximity less to pubertal males in comparison to adult males, further supporting the idea that lower levels of mounting by pubertal males reflects female rather than male choice (Dixson & Nevison, 1997).

In contrast to the data from macaques, in male savanna baboons, first sexual consortship occurs at approximately eight years of age, two to three years after initial testicular increases are observed (Alberts & Altmann, 1995). Thus, the expression and development of sexual behavior in nonhuman primates occurs independently of gonadal hormone changes during puberty as macaques began displaying sexual behavior prior to puberty onset (Alberts & Altmann, 1995; Herman et al., 2006; Nieuwenhuijsen et al., 1988). Despite the effects of maternal rank on puberty onset in male savanna baboons, maternal rank did not influence age at first sexual consortship even after accounting for age when adult rank was attained (Alberts & Altmann, 1995). Thus, earlier puberty onset in male baboons with high-ranking mothers did not result in earlier reproductive success. The time between attaining adult rank and first sexual consortship was approximately 2.5 months, though the data ranged from 5–526 days and this was highly influenced by the number of cycling females in the group (Alberts & Altmann, 1995). First sexual consortship occurred after integration into the male social hierarchy, and therefore, it seems more likely that adult social rank would be a better predictor of age at first consortship, but further research is needed to evaluate this hypothesis.

**Females**

Females being mounted by males was first observed in female stumptail macaques within the first year of life (Nieuwenhuijsen et al., 1988), demonstrating that sexual receptivity, the willingness to be mounted, occurs well before puberty. First copulation occurred between one and three years of age, with first copulation with an adult male occurring between 2.5–3.5 years of age (Nieuwenhuijsen et al., 1988). Age at menarche is not known in this group of outdoor-socially-housed stumptail macaques, making it impossible to relate the expression of copulatory behavior to puberty onset. Rate of copulations increased about six months prior to first ovulation, which occurred at 3.4–4.2 years of age (Nieuwenhuijsen et al., 1988), possibly due to increasing levels of E2 that occur following puberty onset. Rate of copulation was not related to social rank, though social rank was related to the choice of copulation partners. Regardless of age, high-ranking females, in comparison to middle- or low-ranking females, mated more frequently with high-ranking males (Nieuwenhuijsen et al., 1988). In female stumptail macaques, as in males, the expression of sexual behavior occurred well before puberty, but full copulation was temporally similar to the physiological changes during puberty.

In adult female rhesus macaques, social context alters the expression of sexual behavior in that social rank influences the coupling of gonadal hormones and the expression of sexual behavior (Wallen, 1990, 2001). High-ranking females will engage in consistently high levels of sexual behavior throughout the first half of the ovarian cycle - when E2 levels are low but increasing - demonstrating that sexual behavior is not tightly coupled to estradiol levels. In contrast to high-ranking females, low-ranking females show a tight coupling of behavior and E2 levels and only engage in sexual behavior around the time of the midcycle estradiol peak, when motivation is high enough to exceed any social consequences that occur as a result of
mating. Thus, social rank alters the relationship between the expression of sexual behavior and gonadal hormones in adult females (Wallen, 1990, 2001).

In pubertal female rhesus macaques, female sexual initiation appeared to be influenced by social rank in that high-ranking females exhibited the highest rate of female sexual initiation at 2.5 years of age, the age at menarche, regardless of whether or not first ovulation occurred that season (Wallen & Zehr, 2004). In contrast to high-ranking females, middle-ranking females that ovulated at 2.5 years of age showed variability in the levels of sexual initiation observed. One middle-ranking female did not display any sexual initiation, whereas another female showed sexual interest, but levels of female sexual initiation were less than high-ranking females. None of the low-ranking females ovulated or engaged in sexual behavior at 2.5 years of age (Wallen & Zehr, 2004; Zehr et al., 2005). Although the expression of female sexual initiation occurs around the time of puberty, this expression of behavior was not clearly related to the occurrence of menarche or first ovulation. Rather, the expression of sexual initiation appeared to be influenced by social rank, presumably with social rank influencing the level of estradiol required for female sexual initiation to occur. In females that ovulated at 2.5 years of age, there was an increase in female sexual initiation at 3.5 years of age in comparison to the levels of initiation observed a year earlier, when first ovulation occurred (Wallen & Zehr, 2004). At 3.5 years of age, high-ranking females displayed the highest level of sexual initiation and for longer time periods in comparison to middle- and low-ranking females. As in adult females, in pubertal females, middle- and low-ranking individuals displayed sexual initiation, but only around the time of peak E2 levels prior to ovulation (Wallen & Zehr, 2004).

It is possible that the effect of social rank on sexual behavior in pubertal females is a result of aggression from the male. Adult males were more likely to show aggression towards pubertal females and mounting seldom occurred (Wallen & Zehr, 2004) and thus, middle- and low-ranking females may not initiate sexual behavior because the male is unwilling. However, the effects of social rank on sexual behavior expression are similar for adult and pubertal females and therefore, it is not likely that the behavioral effects seen in pubertal females are solely the result of male aggression. It is more likely that social rank modifies sexual behavior by altering the importance of the relationship between estradiol and the expression of sexual behavior. For example, low-ranking females will only engage in sexual behavior for a few days when E2 is high and thus, sexual motivation is high, whereas high-ranking females will engage in sexual behavior throughout the follicular phase, when E2 is low but increasing (Wallen, 1990, 2001). Although the expression of sexual behavior and neuroendocrine puberty are independent processes in female rhesus macaques, the effects of social rank on behavior and motivation likely result in changes in sexual behavior expression at the time of puberty.

In young peripubertal rhesus macaque females, mean age 3.8 years of age, E2 levels were significantly higher 10–30 days prior to the first ovulation of that season in comparison to adult females, 6 years of age or older (Wilson et al., 1982). However, E2 levels did not differ between peripubertal and adult females for the ten days leading up to the first E2 peak of the season. Thus, the longer exposure to greater E2 levels in younger females did not influence E2 levels leading up to the E2 peak or the magnitude of the E2 peak. These younger females also showed a longer period of copulatory activity in comparison to adult females, which is likely a result of the longer increase in E2 levels as this difference in behavior was restricted to the follicular phase of the menstrual cycle. Despite the longer period of observed sexual behavior in young females, there was no difference in the rate of copulatory behavior or births as a function of age. Thus, age only impacted the length of the copulatory period and these age differences in copulatory behavior were not a result of differences in social rank (Wilson et al., 1982).
In summary, the expression of sexual behavior appears to be independent of specific pubertal events in both males and females. Mate choice as well as distinct behavioral patterns likely alter the expression of sexual behavior observed around puberty. Social rank does not influence sexual behavior prior to puberty, but influences behavior during puberty in both males and females. The effects of social rank on sexual behavior during puberty are likely a result of access to mates, though differences in sexual behavior as a result of rank cannot be discounted.

Conclusion

Puberty reflects an integration of the hormonal and social history of an individual and is modulated by the current environmental and social context. Thus, there are many points during development when events later affect the timing of puberty onset or the speed with which pubertal changes occur. The timing of puberty is also one of the more consistent sex differences, with females on average showing earlier puberty onset than do males. Interestingly, the neuroendocrine mechanisms involved in the timing of puberty appear to be sensitive to androgens (both prenatal and neonatal) in males, but do not appear to be sensitive in females. In contrast, leptin appears to influence pubertal events in females, but not in males. Why there would be this asymmetry in response is unclear.

The dramatic changes in sexual behavior that occur around puberty in both males and females raise the possibility that the pubertal elevation in gonadal hormones organizes adult sexual response patterns (Schulz et al., 2009), the result of which is that behavioral patterns that were previously expressed independently of the animal’s hormonal state now come under the control of the activational effects of gonadal steroids. For example, male rhesus monkeys routinely display mounting behavior as juveniles when their testes are nonfunctional. However, after puberty, castration or suppression of testicular function reduces mounting, which is only restored when T levels are increased (Wallen, et al., 1991, Chambers and Phoenix, 1983), even though androgens are not necessary for the display of the mounting motor pattern (Wallen, 1990). Social rank appears to influence sexual behavior in males and females, beginning at the time of puberty (Herman et al., 2006; Wallen & Zehr, 2004). Possibly what is organized at puberty is hormonal modulation of sexual behavior such that it is displayed in relation to the social context and coordinated such that it increases reproductive success.

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Highlights

- In nonhuman primates, social and environmental factors influence the timing of puberty.
- Males and females differ in how these factors influence pubertal events.
- Sexual behavior increases at puberty and may be independent of neuroendocrine change.
- Social context and neuroendocrine function modulate sexual behavior at puberty.
Figure 1.
Primary (solid line) and secondary (dashed line) mechanisms of the hypothalamic-pituitary-gonadal axis feedback loop in (a) male and (b) female nonhuman primates.
Figure 2.
The rate of double-foot clasp mounting by male rhesus macaques during their juvenile and peripubertal time in relation to the sex of the partner. Males mount male and female partners almost equally as juveniles, but markedly increase their mounting of females peripubertally (Adapted from Wallen, 2001).
Figure 3.
The rate of double-foot clasp mounting by male rhesus macaques during their juvenile and peripubertal time in relation to the sex of the partner and whether they were observed to show the ejaculatory reflex during the peripubertal time. Males did not display the ejaculatory reflex as juveniles, but are categorized based on their peripubertal behavior. Only males who had been observed to ejaculate with a female showed a marked increase in mounting of females (Adapted from Wallen, 2001).