American Journal of Primatology 75:212–221 (2013)

REVIEW ARTICLE

The Role of Androgenic Steroids in Shaping Social Phenotypes Across the Lifespan in Male Marmosets (*Callithrix spp.*)

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Steroid hormones, particularly androgens and their metabolic derivatives, play a prominent role in shaping morphological, behavioral, and social phenotypes in many organisms, including primates. This paper reviews the endocrine correlates of development in male marmoset monkeys of the genus *Callithrix* (*C. kuhlii* and *C. geoffroyi*). A lifespan developmental perspective is adopted, in which our knowledge of hormone effects and profiles from prenatal periods through old age is described. Prenatal steroid hormones appear to play a prominent role in shaping behavioral and morphological phenotypes both the prepartum and in the early postpartum periods of life, with exposure to high gestational androgen associated with reduced fetal growth and lower levels of juvenile play. Early postnatal elevations in androgen levels in males are ubiquitous in *Callithrix*, and play a role in the further differentiation of male genital morphology and behavior. Changes in androgens as males approach puberty are similar to the conventional primate pattern, and unlike in female marmosets, gonadal steroidogenesis appears to be independent of social context. In adults, androgens appear to be an important modulator of paternal responsiveness to infants, since androgens are low at times when males typically engage in maximal levels of care, and fathers that care for offspring extensively appear to have lower androgen levels than fathers that are less involved in offspring care. Finally, aging in male marmosets is associated with reduced androgen levels. This reduction appears to be attributable to deficits in central mechanisms, since experimental induction and inhibition of gonadal steroid synthesis and release appears to be normal in older males. Together, these results suggest a complex picture of lifetime involvement of androgens in shaping marmoset phenotypes. Am. J. Primatol. 75:212–221, 2013.

Key words: reproduction; marmoset; androgen hormones

INTRODUCTION

In the vast majority of developmental processes, hormones play prominent and important roles in initiating, maintaining, and canalizing these processes. Most obvious in this regard is the role of gonadal steroids in regulating reproductive biology and behavior. Decades of work on human and nonhuman primates (especially Old World cercopithecine primates) has highlighted the role of androgen and estrogen hormones in shaping genital morphology, masculinizing brain nuclei that regulate sociosexual behavior, and activating sociosexual behavior at the appropriate time of the reproductive cycle and in appropriate seasons of the year [Goy, 1981; Goy et al., 1988; Smith et al., 2013; Wallen, 2005; Wallen & Hassett, 2009]. It is clear from this work that gonadal hormones affect multiple morphological, social, and behavioral phenotypes, but do so in different ways and at different times throughout the developmental lifespan.

In this paper, I will review the role of gonadal steroids in shaping phenotypic traits in marmoset monkeys. These callitrichine primates are interesting species against which to compare the general rules of hormone–behavior relationships in primates because many aspects of their social and reproductive biology contrast sharply with the general primate, and indeed, mammalian, norms of development. Among the distinctive features displayed by marmosets are the following: (1) general lack of body size and pelage dimorphism between the sexes, (2) the obligate production of twin (dizygotic) litters,

Contract Grant Sponsor: National Institutes of Health; Contract grant number: HD042882; Contract Grant Sponsor: National Science Foundation; Contract grant number: IBN 00-91030.

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Received 13 May 2012; revised 31 July 2012; revision accepted 5 August 2012

DOI 10.1002/ajp.22077
Published online 19 September 2012 in Wiley Online Library (wileyonlinelibrary.com).

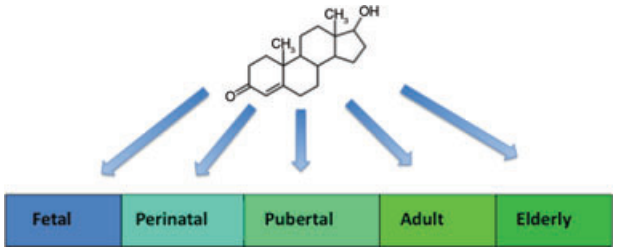

Fig. 1. Lifespan perspective on the impact of androgenic hormones (e.g., testosterone) on morphological, behavioral, social characteristics in male marmoset monkeys.

(3) extensive vascular connections between co-twins in utero, leading to the possibility of close hormonal communication among littermates, (4) the development and maintenance of a strong social bond between adult males and females, (5) the expression of alloparental care by older offspring in natal family groups, and (6) socially mediated reproductive suppression, particularly in daughters and socially subordinate female group members. The focus will be on two species of marmosets of the genus *Callithrix*, Wied's black tufted-ear marmoset (*C. kuhlii*) and white-faced marmosets (*C. geoffroyi*). As this review will highlight, many of the traits that are characteristic of marmoset social and reproductive biology are causally linked to, or associated with, variation in gonadal steroid function. The review will take a lifespan approach to development, and focus on five distinct phases of development both before birth and after parturition, adopting a "womb-to-tomb" perspective (Fig. 1).

GENERAL COMMENTS ON METHODS

Since 1991, the Callitrichid Research Center at the University of Nebraska at Omaha has housed marmoset monkeys in breeding enclosures, with the dual purpose of generating basic knowledge on the biology and behavior of these neotropical primate and participating in captive breeding efforts with North American zoological parks. Marmosets are maintained in normal social groups (breeding pairs with multiple sets of offspring) in large enclosures containing branches, vines, nest boxes, and other locomotor and foraging devices designed to simulate and stimulate naturalistic behavior. Offspring are left in their natal family group through at least two, and possibly more, sets of younger siblings to provide them with exposure to, and experience with, infant-care patterns. Further details of husbandry and housing can be found in [Schaffner et al., 1995].

Endocrine measures are conducted using noninvasive sampling methodology, in which animals are trained, via operant conditioning, to come to the front of their enclosures at the onset of the light phase of the day–night cycle and urinate into aluminum pans in exchange for a desired treat. This training commences as soon as the infants are becoming independent of their caregivers, and involves no restraint or stress. Hormone content in urine samples is assayed by enzyme immunoassays (EIA), and is adjusted for variable fluid intake and output after correction for urinary creatinine concentration. For details on urine-collection protocols, assay procedures, and procedural validation, see [French et al., 1996; Nunes et al., 2002, 2000, 2001].

PRENATAL GONADAL STEROIDS AND DEVELOPMENT

While a good deal of experimental work has been conducted in nonhuman primates on prenatal hormone exposure and later developmental outcomes, the vast majority of this work has been conducted on macaques of the genus *Macaca* [Wallen & Hassett, 2009]. This body of work clearly demonstrates that many features of behavioral, social, and sexual maturation are determined, to a large extent, but not exclusively, by whether or not a fetus or neonate is exposed to androgenic hormones. To my knowledge, no published data exist on normative patterns of hormone levels in fetal male and female marmosets, in spite of the interesting and exciting possibility that because female co-twins share a uterine environment and extensive vascularization with male littermates [Benirschke, 1995], they may be exposed to high levels of testicular hormones secreted by their male wombmate and hence run the risk of prenatal masculinization. Further, no experimental data on prenatal endocrine manipulation have been published (e.g., supplemental androgens, androgen receptor blockers, or steroid synthesis inhibitors). These research strategies have the potential to provide significant insight into both generalized processes of sexual differentiation in marmosets, and to highlight unique features of this process in marmoset monkeys.

We have begun to investigate the impact of normative variation in prenatal exposure to androgens of maternal origin on developmental trajectories in marmosets, recognizing that maternal phenotypic "engineering" is an important source of developmental differences [Maestripieri & Mateo, 2009]. Hormone levels throughout pregnancy in female marmosets are quantified by collecting urine samples noninvasively several times each week, and using competitive hormone binding assays to determine hormone concentrations. Our testosterone antibody utilized in these assays obviously recognizes excreted testosterone concentrations, but also cross-reacts with dihydrotestosterone (DHT) and androstenedione (A4) [Dloniak et al., 2006]. These represent both aromatizable and nonaromatizable androgens, and we refer to our quantitative measures as "urinary androgens" to reflect the promiscuity of the testosterone antibody for multiple forms of androgens. We have demonstrated [French et al.,

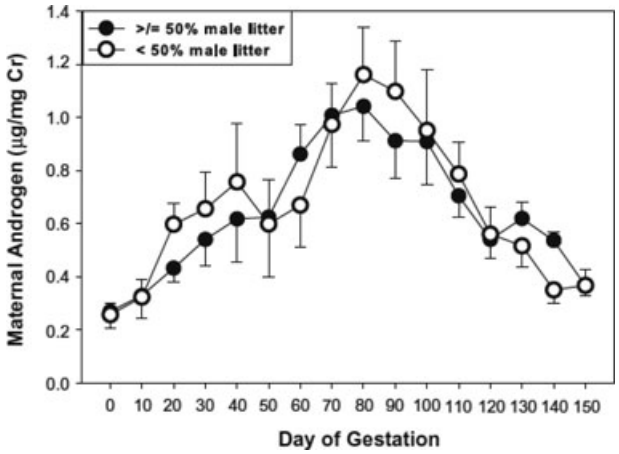

Fig. 2. Changes in maternal androgen across gestation in female white-faced marmosets (*Callithrix geoffroyi*). Dark circles represent females carrying litters that were at least 50% male offspring; open circles represent females carrying litters that were less than 50% male offspring. In this and subsequent figures, vertical bars indicate SEM. From French et al. [2010].

2010] that levels of excreted androgens in female marmosets rise substantially during the first trimester of pregnancy, peak in the second trimester, then gradually decrease during the third trimester (Fig. 2). That these androgens are of maternal (and not fetal) origin is suggested by the following observation: levels of androgen in pregnant females are not associated with either the number of males gestated by the female, nor by the proportion of males in the litter (two related measures of the potential "dose-effect" of male fetuses on maternal endocrinology). Further, neither litter size nor maternal age/parity predicts gestational androgen levels in pregnant female marmosets [French et al., 2010]. We thus conclude that differences in androgen excretion represent variation in production of androgens of ovarian and/or placental origins.

Our group has also demonstrated significant between-female variation in gestational androgen excretion, such that mean gestational androgen excretion in the second trimester can vary as much as threefold among female white-faced marmosets [Smith et al., 2010]. A finding that is equally compelling is the observation that within-female variation in gestational androgen excretion can be even greater than differences among females: second-trimester androgen levels can vary fourfold or more across different pregnancies within the same female. While the source of this variation has not yet been identified (but, as indicated above, is NOT associated with litter size or the presence or absence of male fetuses), these differences are strongly associated with distinct developmental trajectories for both morphological and behavioral measures. Smith et al., [2010] show that morphological growth during gestation is associated with variation in the fetuses' likely exposure to maternal androgen. A variety of morphological measures, including body weight, suprasternal-pubic length, chest circumference, and knee-to-heel length, is significantly smaller at birth in fetuses exposed to higher levels of gestational androgen. For many of these measures, however, growth rates during adolescence were positively associated with maternal gestational androgen. This pattern suggests a biphasic effect of androgen on offspring growth, such that fetal growth is inhibited by androgen exposure, but postnatal growth, particularly once the infant is weaned and is dependent upon solid food to fuel growth, is accelerated by exposure to androgens. Smith et al. [2010] suggested that elevated androgens may allow females to minimize investment in fetal growth, which is especially important since the cost of gestation, relative to other primates, is exceedingly high in marmosets [French et al., 2008]. Androgen-dependent metabolic processes during postnatal development, however, may allow these offspring to show enhanced rates of somatic growth and catch up to those offspring that were exposed to lower gestational androgen and were larger at birth than their high-androgen-exposure counterparts. Experimental studies on androgen manipulation during the prenatal period would provide more definitive evidence regarding the relationships among androgen exposure, metabolic programming, and postnatal growth.

Gestational androgen exposure is associated with behavioral development in marmosets as well. Play patterns in marmosets track the ontogenetic changes seen in other primates [Pereira & Fairbanks, 2002], in that levels of play peak in the adolescent phase [Stevenson, 1977]. In rhesus monkeys, exposure to experimentally elevated gestational androgen in females causes higher rates of rough-and-tumble play, a male-typical play pattern [Goy et al., 1988]. Levels of play in white-faced marmoset juveniles are associated with natural variation in gestational androgen exposure, but in the opposite direction predicted by work on the polygynous macaque monkey. Levels of play with male play partners in female marmosets that experienced high gestational androgen environments were lower than in females exposed to lower gestational androgen [Birnie et al., 2012]. While the specific neural mechanisms associated with lower rates of play after high androgen exposure have not been identified, the lower rates of energetically costly play behavior are consistent with the allocation of resources toward somatic growth in offspring that experienced high gestational androgen exposure.

PERI- AND EARLY POSTNATAL GONADAL STEROIDS

Like most mammals, male marmosets exhibit high levels of androgens in the early postpartum

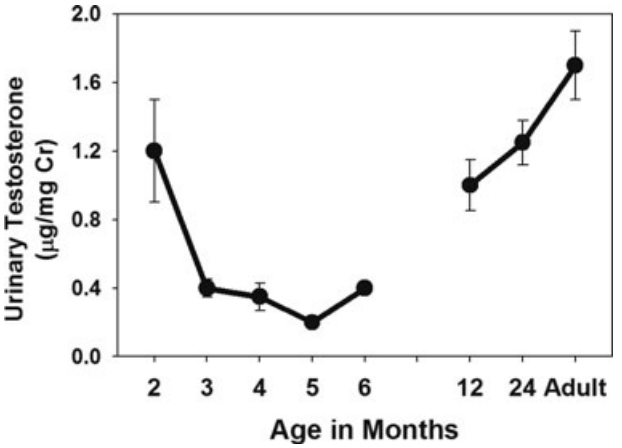

Fig. 3. Changes in urinary androgen excretion in male black tufted-ear marmosets (*C. kuhlii*). Adapted from French and Schaffner [1995].

period. Figure 3 portrays levels of urinary steroid in male black tufted-ear marmosets during months 2–6 of life (relative to average levels in breeding adult males). While older infants have low and unremarkable concentrations of urinary androgen excretion, 2-month-old marmosets display high levels of androgen excretion, levels that are not statistically distinguishable from those of breeding adult males [French & Schaffner, 1995]. In a similar fashion, male common marmoset (*C. jacchus*) also have high levels of circulating testosterone in the immediate postpartum period, staying elevated for 1–2 months [Abbott & Hearn, 1978b; Dixson, 1986; Mann & Fraser, 1996]. The presence of this early postnatal peak in androgens has led to the suggestion that early postnatal androgen production may be critical for morphological and behavioral masculinization of male marmosets [Abbott & Hearn, 1978a]. This suggestion has merit because, similar to other species like rodents that give birth to multiple offspring of both sexes within a single litter, a postnatal period of sexual differentiation in marmosets may be an adaptation that minimizes the possibility of prenatal masculinization of female fetuses by male co-twins. Two experimental studies support this notion of a postnatal sensitive period for sexual differentiation of both behavior and morphology in marmosets. Postnatal testosterone treatment masculinizes female marmoset genitalia, with hypertrophy of the clitoris and enlargement of the labia to resemble scrotal folds. As adults, treated females exhibit both high levels of aggression toward conspecific males and sexual behavior toward females [Abbott, 1984b; Abbott & Hearn, 1978a]. Conversely, neonatal castration of male offspring produced adult males with small genitalia, and testosterone treatment of these adults produced an increase in penile and scrotal size. Castrating neonatal males also leads to reduced sexual behavior with receptive females and reduced aggression toward unfamiliar males in adulthood [Dixson, 1993]. The complementary sets of studies on male and female marmosets suggest an important postnatal sensitive period for differentiation, and these outcomes are certainly the obvious sequelae of elevated postnatal androgen concentrations in male marmosets.

PERIPUBERTAL GONADAL STEROIDS

Female callitrichine primates have served as excellent models for the social modulation of the onset of reproductive function, since social context plays an exceedingly important role in regulating the timing of both ovarian maturation, normative reproductive function, and the expression of female sexual behavior. Our appreciation of the ways in which social environments affect female ovarian function has evolved considerably from early reports suggesting a simplistic relationship: to wit, all daughters and/or subordinate females were reproductively inhibited by ovarian suppression [Abbott, 1984a; French et al., 1984]. There is now a greater appreciation for the notion that while subordinates and daughters suffer reduced reproductive function and reduced sexual behavior, reproductive suppression is not a universal characteristic of subordinates and daughters (see reviews in [French, 1997; Saltzman et al., 2009; Ziegler & Sousa, 2002], a number of variables interact to determine whether and at what age subordinates undergo sexual maturation and express ovulatory ovarian cycles.

Less attention has been paid to the onset of reproductive function in male marmosets (sons and subordinates) living in the presence of mothers and fathers or in groups with unrelated breeders. Abbott and Hearn [1978b] and Baker et al. [1999] report that sons living in family groups have slightly, but not significantly, lower levels of circulating testosterone than their adult-aged fathers. We recently conducted an extensive longitudinal assessment of urinary androgen excretion in male white-faced marmosets living in their natal family groups [Birnie et al., 2011], and specifically addressed the question of whether sons display any degree of suppression of androgen levels while in their natal family group. Androgen levels rise throughout development in males, from infancy to juvenile to subadult to young adult (Fig. 4). Although sons have androgen concentrations that are, on average, 20–30% lower than adult-aged fathers, these differences did not reach conventional levels of significance. Thus, in spite of prominent, and in some cases, dramatic effects of social environments on female marmoset ovarian function, gonadal production of androgens in males appears to be relatively insensitive to social context.

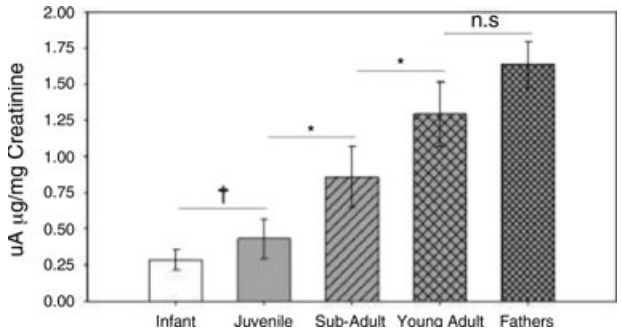

Fig. 4. Developmental trends in androgen excretion in male white-faced marmosets (*C. geoffroyi*). From Birnie et al. [2011].

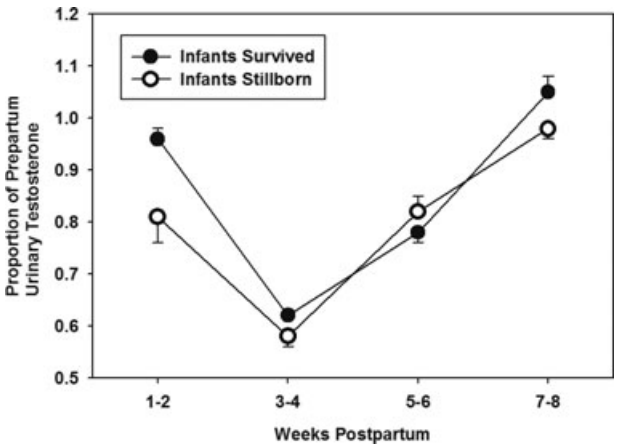

Fig. 5. Proportionate changes in urinary androgen excretion in male marmosets (*C. kuhlii*) during the first 8 weeks after the birth of offspring, relative to androgen excretion in the 2 weeks prepartum. Dark circles represent androgen levels in fathers whose infants survived, open circles represent androgen levels in fathers whose infants were stillborn (and hence fathers engaged in no active paternal care). Modified from Nunes et al. [2000].

GONADAL STEROIDS AND PATERNAL BEHAVIOR IN ADULTHOOD

The primary focus of the Callitrichid Research Center vis-à-vis androgens and adult social phenotype has been on the role that androgens may play in modulating the inevitable trade-offs between sexual effort and parental effort in males. As indicated earlier in this review, marmosets represent one of the few groups of primates that exhibit high levels of paternal care of offspring, which requires nurturant, tolerant sociality to enhance the survivorship of current offspring, a strategy that is potentially antithetical to aggression and sexual behavior that could contribute to future reproductive success for male marmosets.

We have evaluated the androgen correlates of changes in male paternal behavior in these realms that may underlie the important trade-offs between sex and paternal care. First, what kind of care do males provide for offspring, and when do they perform these activities with dependent offspring? Our observational data show three distinct phases of male offspring care. In the first week or two after the birth of infants, the typical marmoset father (in both Wied's black tufted-ear marmosets [Nunes et al., 2000, 2001] and white-faced marmosets [Cavanaugh & French, unpublished data]) is exceedingly motivated to interact with newborn offspring, but is less involved in carrying offspring than is the mother. During the second 2 weeks postpartum, fathers become the primary caregivers for infants during the active daylight hours, carrying the infant more than 50% of the time (Mothers continue to be primary caregivers to infants throughout development during night-time hours, and have significantly more disrupted sleep than any other family member [Fite et al., 2003]. After the infants reach 4 weeks of age, fathers, like all other caregivers in the family, reduce infant-carrying effort gradually as the infants become more independent and are weaned at 8–12 weeks of age. This inverted U-shaped pattern of paternal care during early infant life is strongly associated with temporal changes in androgen levels in fathers (Fig. 5; Nunes et al. [2000]). Levels of androgens are slightly lower than prepartum values during the first 2 weeks postpartum, but then drop dramatically by 40% during the 2-week period during which fathers are primary daytime caregivers. Levels begin to return to normal prepartum levels as the infants reach independence. These data are consistent with the notion that low androgen levels are compatible with high levels of offspring care, but clearly are not conclusive. We have since replicated these general patterns in white-faced marmoset males as well, who show low androgen and elevated paternal care during weeks 3 and 4 postpartum [Cavanaugh & French, unpublished data]. Figure 5 also presents the important finding that androgen levels in fathers whose mates give birth to stillborn infants (and hence fathers do not engage in paternal care) show identical patterns of decrease in androgen that we document in fathers that care for offspring during the postpartum period. This finding is important for two reasons. First, it suggests that cues associated with the mate's pregnancy and/or peripartum stimuli are important triggers for endocrine changes, since both sets of fathers show similar patterns of endocrine change. Second, this result provides additional evidence that the changes we have documented in androgen production are more likely to be a cause of, rather than a consequence of, long-term exposure to and interaction with infants in the postpartum period.

Supporting evidence for a role for androgens in paternal responsiveness comes from an individual-differences analysis; that is, fathers that exhibit high levels of paternal care in the postpartum period have androgen levels that are significantly lower than fathers who are less involved in offspring care

[Nunes et al., 2001]. Together with evidence that male common marmosets show reduced androgen levels following exposure to infant-related stimuli [Ziegler, 2013], the pattern of results from black tufted-ear and white-faced marmosets indicates that androgens may be important mediators of paternal responsiveness to, and care of, dependent offspring. Relatedly, we have also reported that female marmoset maternal effort is associated with androgen levels, such that females reduce maternal effort early in the postpartum period as their androgen excretions levels rise [Fite et al., 2005]. This parallel finding in both males and females provides converging correlational evidence that the role of androgens in shaping infant caregiving phenotypes is similar in both sexes, presumably operating on similar neural substrates of parental motivation and infant tolerance.

AGING AND GONADAL STEROIDS IN MALE MARMOSETS

The decline and cessation of normative reproductive function across aging in a few species of female primates (including human females) has been well documented (see reviews in Broekmans et al. [2007]; Schramm et al. [2002]). Among male primates, the most ubiquitous effect of age on reproductive function is the gradual but consistent reduction in testosterone with increasing age. This reduction with age is brought about by multiple changes in the operating characteristics of the hypothalamic–pituitary–gonadal (HPG) axis. The most prominent of these changes include a reduction in the magnitude and number of gonadotropin-releasing hormone (GnRH) pulses from hypothalamic nuclei, a decrease in the responsivity to luteinizing hormone (LH) in steroid-synthesizing Leydig cells in the testes, and a greater negative feedback effect of circulating testosterone on GnRH synthesis and release [Keenan et al., 2006].

Aging has the expected effect on offspring production in female marmosets: females show higher rates of anovulation as they approach the average maximum lifespan. The single published study on aging and reproductive function in female marmosets (and tamarins: Tardif and Ziegler [1992] found higher rates of anovulatory ovarian cycles in aged females than in younger adult females, which was associated with a reduction in both total follicular number and fewer large preantral follicles in aged females. Surprisingly, however, ovarian steroid hormone concentrations in aged females were as high as, or higher than, those measured in younger females. So, in spite of decrements in ovulatory efficiency, interstitial ovarian cells maintain steroidogenic properties in older females and endocrine levels do not appear to decrease significantly with age. Given this surprising *lack* of an effect of aging in female marmosets, we began a systematic study of changes in androgens in male marmosets across the lifespan.

Two strategies comprised our approach to aging effects in male marmosets (*C. kuhlii*). We first surveyed hormone concentrations in males across a wide swath of adult ages (2–15 years of age) to document any potential decreases in male androgen production over time. Second, we assessed function in the HPG axis in two ways: by conducting a GnRH-stimulation experiment (which can assess both pituitary gonadotroph and testicular responses) and by administering a GnRH antagonist to males of differing ages.

Androgen Profiles Across Age

We have previously published data on androgens across the lifespan in male marmosets [Tardif et al., 2008]. We accessed archived urine samples from 15 males, who each provided data throughout their lifetimes in the Callitrichid Research Center. Most males contributed samples through 12 years of age, and one male was sampled beyond 14 years of age. A scatterplot of androgen concentrations by age is shown in Figure 6 (top panel), and several features of the pattern are worth noting. First, there is considerable variation in androgen levels in male marmosets at any given age, and although some of the sources of the variance have been identified earlier in this review (e.g., paternal status), much of the variation remains unexplained. Second, there is a statistically detectable, but subtle, effect of age on androgen levels in males. A best-fitting polynomial function (shown in Fig. 6, top panel) reveals a curvilinear relationship between age and androgen. Levels rose in adult males to about 7 years of age, at which time there was an inflection point in the relationship and androgen levels declined thereafter. A more detailed analysis of 6-month averages (which tends to minimize day-to-day variation) in males from 6 to 15 years of age is shown in Figure 6 (bottom panel), where the relationship is clearer—there is a small but significant decrease in androgen excretion as male marmosets age, similar to other primates, including human beings. Like other primates, including humans, male marmosets show decreases in androgen levels as they age.

HPG Function Across Age in Males

The approach to evaluating changes in HPG function across age involved treatment with a GnRH challenge [Nunes et al., 2002] and GnRH antagonists [Armstrong, 2006]. The first technique allows us to assess Leydig cell function across age directly by assessing differences in androgen production after stimulation in younger vs. older males, and pituitary gonadotrope function indirectly, in the sense that any increases in androgen after GnRH

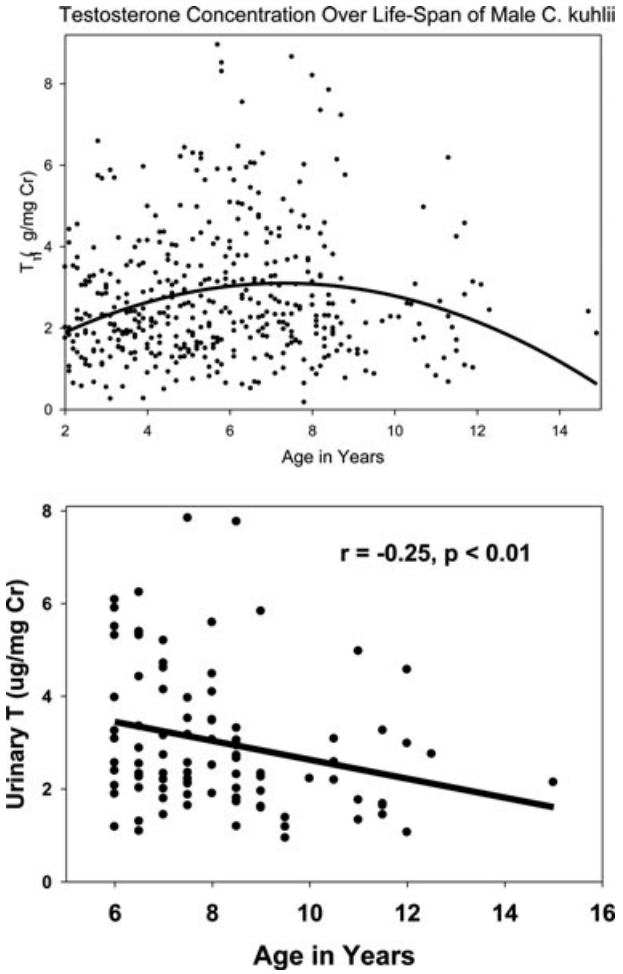

Fig. 6. Top panel: changes in urinary androgen from 2 to 15 years of age in adult male marmosets (*C. kuhlii*). Dark line represents best-fitting polynomial regression between age and androgen levels. Bottom panel: mean 6-month average androgen excretion rates for male marmosets from 6 to 15 years of age.

stimulation must result from enhanced pituitary release of LH. Evaluation of androgens following GnRH antagonist treatment allowed for inferences of hypothalamic sufficiency, since antagonist treatment in older males with low endogenous GnRH activity would lead to little or no change in androgen production. On the other hand, if GnRH release was adequate in older, as well as younger males, we would predict reductions in androgen production in both sets of males after GnRH antagonist treatment.

GnRH challenge

Twelve male marmosets were evaluated in our study [Nunes et al., 2002; Tardif et al., 2008], eight of whom received GnRH (Sigma Chemical, St. Louis, MO, USA; 2 μg intravenous [i.v.]) while the other four received i.v. saline vehicle. Plasma androgens were evaluated in samples collected immediately prior to and 45–60 min after treatment. All

Fig. 7. Proportionate change in plasma androgen concentrations after GnRH challenge as a function of age in male marmosets (*C. kuhlii*).

GnRH-treated males showed increases in plasma androgens, while androgens in vehicle-treated males did not change after treatment. Among the treated males, however, age was a significant predictor of the magnitude of increase in circulating androgens (Fig. 7). Older males recruited a significantly larger increase in circulating androgens than younger males, with some of the oldest males exhibiting a four- to sixfold increase in circulating androgen. Patterns of plasma estradiol followed the same pattern: GnRH-treated males had elevated circulating estradiol relative to saline-treated males, and older males showed proportionately greater increases in estradiol following the GnRH challenge. These findings indicate that Leydig cells in older male marmosets, like steroidogenic interstitial ovarian cells in female marmosets, maintain their capacity to synthesize and release androgen. Further, and by implication, they suggest that the aging male marmoset pituitary remains sensitive (and possibly hypersensitive) to stimulation from hypothalamic GnRH in the release of LH.

GnRH antagonist

To test the effects of GnRH antagonism on function in the HPG axis, we selected a "new-generation" GnRH antagonist, acyline [Herbst et al., 2002; Jiang et al. 2001]. In our study [Armstrong, 2006], we selected 14 adult males that ranged in age from 3 to 15 years. All animals received two treatments in a counterbalanced order, with treatments separated by several weeks: five consecutive daily 25 μg/kg intraperitoneal (i.p.) administrations of acyline or an equivalent volume of saline vehicle. In this study, testicular testosterone production was estimated by monitoring urinary androgen levels. Acyline treatment was clearly effective in the inhibition of androgen synthesis and release: relative to saline-treated males, acyline-treated males showed

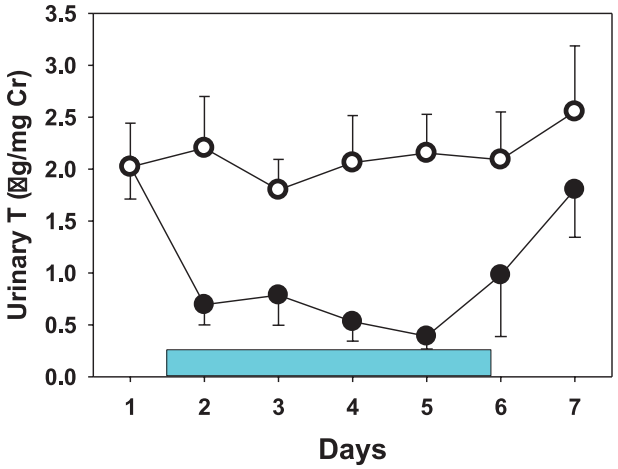

Fig. 8. Androgen excretion levels in male marmosets (*C. kuhlii*) after treatment with GnRH antagonist acyline (dark circles) vs. saline vehicle (open circles). Solid bar on *x*-axis represents days of acyline treatment. There is a significant effect of treatment ($F(1,24) = 13.54$, $P < 0.01$), with acyline-treated males showed lower excreted androgens on days 2–6 of the study ($P < 0.05$). Unpublished data from Armstrong [2006].

markedly reduced levels of androgen excretion, with levels approximately 75% lower during acyline treatment than during saline treatment (Fig. 8). As in the GnRH agonist study, male age was a significant predictor of the magnitude of androgen suppression by acyline. Figure 9 shows that older males had significantly lower excreted androgen levels after acyline treatment when compared to younger adult males. As with the GnRH agonist data, these results provide further confirmation that steroidogenic Leydig cells within the testes of aging male marmosets continue in their capacity to respond to pituitary gonadotropins with sufficient synthesis and release of androgens, and strongly suggest that changes in androgens as male marmosets age are associated with

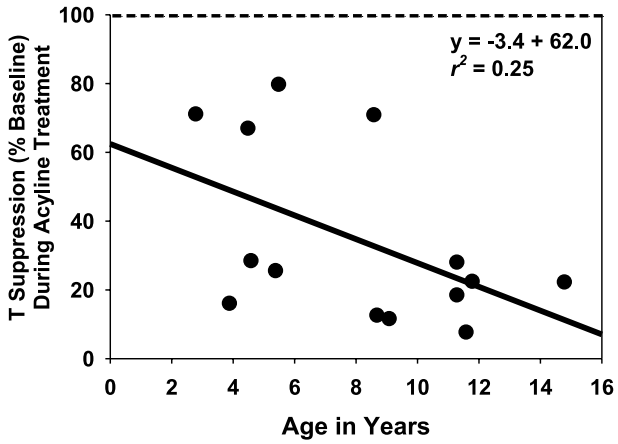

Fig. 9. Age-related changes (expressed as a function of pretreatment excreted androgen concentrations) in male marmosets treated with the GnRH antagonist acyline.

alterations in hypothalamic and pituitary function in the HPG axis. Further elucidation of the specific neuroendocrine mechanisms that underlie reduced androgen production, secretion, and excretion in male marmosets requires more detailed mechanistic examination of hypothalamic GnRH and pituitary gonadotropin dynamics.

CONCLUSIONS

This review highlights the important ways in which androgens appear to guide, canalize, activate, and shape male marmoset reproductive biology and behavior. Early androgen exposure, either during the prenatal period or immediately after birth, has an impact not only on the masculinization of external genitalia, but also on behavioral phenotypes including play, sexual behavior, and aggression. In spite of the lability of female marmoset reproductive function in light of social context, however, male marmoset production of androgen appears to be independent of the social environment in which they are housed. The important callitrichine primate trait of high levels of paternal solicitude toward infants is temporally correlated with changes in androgen production, and individual differences in the degree of involvement with infants during the periods of critical offspring care can be predicted by individually specific levels of androgen production. Finally, male marmosets, in common with female marmosets, share the characteristic that steroidogenic cells in the gonads remain highly responsive to gonadotropins in aging animals, and the gonadal component of the HPG axis does not appear to be dysfunctional in older marmosets. Thus, the data evaluated in this review point out some features that male marmosets share with other nonhuman primates, but also highlight important differences that render marmosets an interesting model for male reproductive and social endocrinology.

ACKNOWLEDGMENTS

Much of the published and original research presented in this paper has been supported by grants from the National Institutes of Health (HD042882) and the National Science Foundation (IBN 00-91030) awarded to JAF. Many graduate students and postdoctoral associates have contributed ideas and data, including Colleen Schaffner, Jeff Fite, Diana Armstrong, Adam Smith, Drew Birnie, and Aaryn Mustoe. The Callitrichid Research Center at the University of Nebraska at Omaha has been ably managed for over 10 years by Heather A. Jensen, and her contributions to the health and well-being of the quadrupedal members of our research team has enabled the bipedal primates to conduct science knowing that the marmosets receive the highest quality care possible. This research adhered to the American

Society of Primatologists principles for the ethical treatment of primates.

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