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Maternal gestational androgens are associated with decreased juvenile play in white-faced marmosets (*Callithrix geoffroyi*)

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ABSTRACT

Exposure to androgens during prenatal development shapes both physiological and behavioral developmental trajectories. Notably, in rhesus macaques, prenatal androgen exposure has been shown to increase rough-and-tumble play, a prominent behavioral feature in males during the juvenile period in primates. While macaques are an Old World, polygamous species with marked sexually dimorphic behavior, New World callitrichine primates (marmosets and tamarins) live in cooperative breeding groups and are considered to be socially monogamous and exhibit minimal sexual dimorphism in social play, which suggests that androgen may affect this species in different ways compared to macaques. In addition, we previously described considerable variation in maternal androgen production during gestation in marmosets. Here we tested the association between this variation and variation in offspring rough-and-tumble play patterns in both males and females. We measured testosterone and androstenedione levels in urine samples collected from pregnant marmoset mothers and then observed their offspring's play behavior as juveniles (5–10 months of age). In contrast to findings in rhesus macaques, hierarchical regression analyses showed that higher gestational testosterone levels, primarily in the second semester, were associated with decreased rough-and-tumble play in juveniles, and this relationship appears to be driven more so by males than females. We found no reliable associations between gestational androstenedione and juvenile play behavior. Our findings provide evidence to suggest that normative variation in levels of maternal androgen during gestation may influence developmental behavioral trajectories in marmosets in a way that contradicts previous findings in Old World primates.

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Introduction

Exposure to androgenic hormones during sensitive periods of development can substantially alter a number of developmental trajectories. This so-called organizational effect of androgens (Phoenix et al., 1959) can account for masculinization/defeminization of genitalia and other secondary sexual characteristics as well as shaping behavioral predispositions. Of the behaviors influenced by early exposure to androgens, social rough-and-tumble play, or play fighting, during the juvenile period is of particular importance due to its proposed role in shaping outcomes related to foraging/hunting, cognition, learning, reproduction, and sociality in mammals (reviewed in Power, 2000). In rats, juvenile males show higher rates of rough-and-tumble play than their female counterparts and androgen exposure during early postnatal life increases rates of rough-and-tumble play in females (reviewed in Thor and Holloway, 1985). Furthermore, in rats it appears that exposure to androgens during both the pre- and early post-natal periods is required for sexually dimorphic play and other sex-typical behaviors to fully develop (Casto et al., 2003). Evidence for prenatal androgen effects on juvenile play behavior in humans is provided by studies showing that girls with congenital adrenal hyperplasia (CAH)—a genetic disorder that causes enzyme deficiencies resulting in excessive androgen production beginning in utero. Girls with CAH tend to prefer male-typical toys during early childhood and adolescence, though no striking differences in rough-and-tumble play behavior have been found between girls with CAH and their normal peers (Berenbaum and Hines, 1992; Berenbaum et al., 2000; Hines, 2003; Hines and Kaufman, 1994; Meyer-Bahlburg et al., 2008; Nordenstrom et al., 2002).

In non-human primates, research on the effects of manipulation of maternal gestational androgens has focused on the rhesus macaque (*Macaca mulatta*). Macaques are a polygynous species that exhibits both physical and behavioral sexual dimorphism. For example, juvenile males initiate more rough-and-tumble play bouts than juvenile females (reviewed in Wallen and Hassett, 2009). Exogenous testosterone (T) propionate given during gestation masculinizes and/or defeminizes both morphological and behavioral development of fetuses in this species (Goy et al., 1988; Thornton et al., 2009), while early postnatal manipulations (without prenatal manipulations) of T have produced

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no striking effects on later juvenile or adult behavior in macaques (Thornton et al., 2009; Wallen, 2005; Wallen and Hassett, 2009). It may instead be that, similar to rats, both pre- and postnatal androgens are necessary to develop a fully masculinized/defeminized behavioral repertoire in macaques as Gibber and Goy (1985) found that prenatally androgenized females showed little behavioral dimorphism from neonatally castrated males as juveniles. Taken together, these results support a masculinizing/defeminizing organizational role of prenatal androgens in the behavioral development of the rhesus macaque similar to that seen in rodents. Thus, exposure to prenatal androgen – particularly during late gestation – can influence sexually dimorphic behavior throughout the primate lifespan.

Callitrichine primates (marmosets and tamarins), on the other hand, exhibit few sexually dimorphic traits (Ford, 1994; Plavcan, 2001). These New World primates typically give birth to fraternal twins and live in socially monogamous, extended family groups with fathers and older siblings providing a significant role in caring for the young (reviewed in Tardif et al., 1993). These features (reduced sexual dimorphism and a cooperative breeding social structure) might portend patterns of gender-based differences in rough-and-tumble play that would deviate from those observed in other polygynous primate species. For example, in squirrel monkeys (Biben, 1998) and rhesus macaques (reviewed in Wallen, 2005), the rate, intensity, and duration of rough-and-tumble play are all higher in males than females. However, the sex differences in rough-and-tumble play in socially monogamous primates are not completely understood. Sex differences in play patterns appear to be subtler, if at all present. For example, in common marmosets (Stevenson and Poole, 1982) and cotton-top tamarins (Cleveland and Snowdon, 1984), there appears to be little, if any, sexual dimorphism in the overall rate of play. Chau et al. (2008), however, did find that while the rate of play did not differ between captive male and female coppery titi monkeys – a socially monogamous, new world primate that also lives in extended family groups – there are sex differences in play partner preferences: juvenile females engage in more rough-and-tumble play with their fathers than do juvenile males. Also, while juvenile marmosets engage in rough-and-tumble play with all members of their extended families, the co-twin is generally the preferred play partner (Stevenson and Poole, 1982). One report suggests that males initiate more rough-and-tumble play bouts with female co-twins whereas male–male co-twin pairs initiate bouts equally (Abbott and Hearn, 1978a). In addition, administration of T early in postnatal life appears to increase rough-and-tumble twin play initiations in female marmosets (Abbott & Hearn, 1978a, 1978b; Abbott, 1984). Thus, in socially monogamous new world primate species, early exposure to androgens may differentially influence play patterns, which could include the rate of rough-tumble-play and play partner preference. However, to our knowledge, no reports have investigated how prenatal exposure to androgen might influence rough-and-tumble play in a socially monogamous species such as the marmoset.

Our laboratory recently reported that urinary testosterone (T) levels rise during the first trimester of pregnancy reaching peak levels during the second trimester and decline in the third trimester in the white-faced marmoset (*Callithrix geoffroyi*; French et al., 2010; Smith et al., 2010). These results parallel previous reports of gestational T and concentrations measured in plasma in the common marmoset (Chambers and Hearn, 1979). Interestingly, we also observed considerable variation in maternal urinary androgen levels across individual females and across different pregnancies of the same female. This variation in androgen levels was not related to fetal sex or number of male fetuses (French et al., 2010). However, it was associated with variations in the offspring's somatic growth: high maternal androgen levels during early gestation were associated with lower growth rates early in postnatal life followed by a subsequent period of catch-up growth during the juvenile period (Smith et al., 2010). The goal of the current study was to characterize the relationship, if any, between these variations in gestational androgens and juvenile rough-and-tumble play.

Androstenedione (A^4) is a steroid precursor to both T and estrogens and has been associated with in utero effects on development in several mammals including the hyena (Licht et al., 1992; Yalcinkaya et al., 1993) and cavy (Kraus et al., 2008). Chambers and Hearn (1979) report high concentrations of A^4 during gestation in the common marmoset. Findings from perinatal administration of A^4 in male rats (Gilroy and Ward, 1978; Goldfoot et al., 1969) and female mice (Edwards, 1971) suggest that this androgen is involved in masculinization but not the defeminization of adult sexual behavior in rodents; i.e., intromission and ejaculation behaviors in males may be retained simultaneously with lordosis following neonatal A^4 treatment.

Given the variation in gestational T observed in our previous studies (French et al., 2010; Smith et al., 2010), the present study sought to evaluate variation in rough-and-tumble play in juvenile white-faced marmosets and maternal concentrations of urinary androgens. To the extent that the behavioral development of marmosets is sensitive to natural levels of androgen exposure in utero, we predicted that higher levels of maternal androgen within normative variation during late gestation of these hormones would be associated with higher rates of rough-and-tumble play in juvenile offspring.

Materials and methods

Subjects

Androgen levels were measured in urine from 6 white-faced marmoset (*C. geoffroyi*) mothers across 18 pregnancies, ranging from 1 to 6 pregnancies per female and resulting in 29 viable offspring (16 males, 13 females). Mothers ranged in age at conception from 2.2 to 7.7 years old. Animals were socially housed in groups of 3 to 9 animals at the University of Nebraska at Omaha Callitrichid Research Center in wire-mesh enclosures no smaller than 1 m × 2 m × 2 m with no less than 1 m^3 per animal. Cages were furnished with branches, a nest box, and various enrichment devices. Animals had access to water ad libitum and were fed Zupreem® Marmoset chow and fiber blocks each day, supplemented with varying combinations of meal worms, crickets, various fruits, yogurt and eggs. Adequate steps were taken to ensure minimal pain and discomfort and all procedures complied with and were approved by the University of Nebraska Medical Center/University of Nebraska at Omaha Institutional Animal Care and Use Committee (IACUC).

Urine collection

We collected one to two urine samples per week from each mother using noninvasive techniques described by (French et al., 1996). Briefly, subjects were trained to urinate into handheld pans for a preferred food item in their home cage. We collected 0.05–1.0 ml of first void urine samples from each animal between 0600 and 0830 h after the animals first woke. Samples were transferred to plastic vials, centrifuged at 7000 rpm for 2 min to remove sediments, and then the supernatant portion of each sample was transferred to a clean vial and stored at −20 °C until assayed.

Enzyme immunoassays (EIAs)

To determine dates of conception and gestation periods, urine samples were evaluated for concentrations of the progesterone metabolite, pregnanediol-3-glucuronide (PdG) using an enzyme immunoassay (EIA). Conception dates were determined retrospectively by estimating the date at which urinary PdG concentrations reached a nadir that was immediately followed by a sharp and sustained rise in urinary PdG concentrations which terminated in pregnancy (French et al., 1996). Three different raters scored their estimates of conception dates independently and inter-rater reliability was over 98% (within 2 days). Levels of urinary creatinine (Cr), a muscle metabolite excreted at near constant rates, were