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Prenatal androgen exposure and children's aggressive behavior and activity level☆

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ABSTRACT

Some human behaviors, including aggression and activity level, differ on average for males and females. Here we report findings from two studies investigating possible relations between prenatal androgen and children's aggression and activity level. For study 1, aggression and activity level scores for 43 girls and 38 boys, aged 4 to 11 years, with congenital adrenal hyperplasia (CAH, a genetic condition causing increased adrenal androgen production beginning prenatally) were compared to those of similarly-aged, unaffected relatives (41 girls, 31 boys). Girls with CAH scored higher on aggression than unaffected girls, $d = 0.69$, and unaffected boys scored higher on activity level than unaffected girls, $d = 0.50$. No other group differences were significant. For study 2, the relationship of amniotic fluid testosterone to aggression and activity level was investigated in typically-developing children (48 girls, 44 boys), aged 3 to 5 years. Boys scored higher than girls on aggression, $d = 0.41$, and activity level, $d = 0.50$. However, amniotic fluid testosterone was not a significant predictor of aggression or activity level for either sex. The results of the two studies provide some support for an influence of prenatal androgen exposure on children's aggressive behavior, but not activity level. The within-sex variation in amniotic fluid testosterone may not be sufficient to allow reliable assessment of relations to aggression or activity level.

1. Introduction

Meta-analytic findings suggest that boys are more aggressive and more active than girls, with Cohen's *d* values of 0.58 and 0.49, respectively (Eaton and Enns, 1986; Hyde, 1984). In humans, aggression is defined as behavior, typically directed toward another individual, that is carried out with the intent of causing harm (Bushman and Anderson, 2001) and activity level is defined as the typical level of energy that an individual expends through movement (Eaton and Enns, 1986). There is general agreement that gender differences in human behavior result from a combination of genetic, early hormonal and socio-cultural influences (Hines, 2015; Leaper, 2013). The current investigation examines the relation of the gonadal steroid, testosterone, prenatally to later aggression and activity level in children.

Prior research with nonhuman animals shows that exposure to the androgenic hormone, testosterone, during critical periods of prenatal or neonatal development exerts enduring influences on many behaviors that show sex differences (Arnold, 2009). These effects occur as part of general processes of sexual differentiation. The testes of male animals produce androgens, including testosterone, beginning prenatally, and these hormones act through steroid receptors to produce male-typical development of the external genitalia. Similar steroid receptors are present in certain brain regions, and these regions also are masculinized by androgen exposure during early life. These early effects of androgens on brain organization are thought to contribute to sex-related behaviors across the lifespan (Arnold, 2009; McCarthy et al., 2009), and experimental manipulations of testosterone during early development have been found to influence reproductive behaviors, as well as juvenile play behavior, aggression, and activity level in rodents (Hines, 2004, 2011a, 2011b; McCarthy et al., 2009).

Regarding aggression, male rodents typically show more aggression

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than female rodents, and treatment with testosterone early in life increases aggression in adulthood (Beatty, 1979; Hines, 2004; vom Saal, 1989). Similarly, early exposure to testosterone influences activity level in rodents (Beatty, 1979; Lightfoot, 2008; vom Saal, 1989). In humans, males are more active than females. In most other mammals, including rodents, however, females are more active than males. In keeping with the direction of this sex difference, early exposure to testosterone in female rodents reduces rather than increases activity level—that is, makes it more male typical (Broida and Svare, 1984; vom Saal, 1989).

The available evidence suggests that testosterone concentrations during early life also influence the development of some human behaviors that differ on average for males and females (Hines, 2011a). Male and female fetuses are exposed to androgens, including testosterone, that are produced primarily by the gonads, but also by the adrenal glands, the placenta and the maternal system (Braunstein, 2003; Fisher, 2003; Stewart, 2003). Testosterone levels are higher in human male than in human female fetuses from around week 8 to around week 24 of gestation (Smail et al., 1981). Because the invasive experimental procedures used to study hormonal influences in non-human animals cannot be applied to humans, alternative methods have been used to assess prenatal hormone influences on human development. One such method is to study naturally occurring situations, such as endocrine disorders, in which hormones are altered. Using this approach, individuals who have been exposed to unusual levels of androgens prenatally, e.g., because of genetic disorders, are compared to individuals who have not been similarly exposed (e.g., unaffected relatives or matched controls). The most frequently studied endocrine disorder, in this context, is congenital adrenal hyperplasia (CAH), an autosomal recessive disorder that causes an enzymatic deficiency and results in overproduction of adrenal androgens beginning prenatally (White and Speiser, 2000). The most common form of the disorder, classic CAH, occurs in approximately 1 in 5000 to 1 in 15,000 births in most populations (New, 1998) and typically involves deficiency in the enzyme 21-hydroxylase (21-OH). The deficiency causes reduced cortisol production and, as a consequence, overproduction of adrenal androgens beginning at around the seventh week of gestation. Because of this prenatal exposure to elevated adrenal androgens, girls with CAH—whose androgen levels at mid-pregnancy are similar to those seen in typically-developing boys—are often born with ambiguous (virilized) external genitalia, involving various degrees of labial fusion and clitoral enlargement. Typically, this genital ambiguity leads to diagnosis soon after birth and sex assignment as female, sometimes with surgical feminization of the external genitalia (Speiser et al., 2010). In contrast, androgen concentrations prenatally in boys with CAH appear to be largely within the normal male range and boys with CAH are born with male-typical external genitalia (Speiser et al., 2010).

Although most studies have found that gender-related behaviors in boys with CAH are not altered (for reviews see Cohen-Kettenis, 2010; Hines, 2015), females with CAH show increases in some male-typical behaviors from an early age. For example, girls with CAH are more likely than unaffected girls to prefer boys as playmates (Hines and Kaufman, 1994) and to prefer boys' toys (e.g., vehicles versus dolls; Berenbaum and Hines, 1992) and activities (e.g., rough-and-tumble play; Pasterski et al., 2011). These outcomes of increased male-typical play have been reported both in comparison to matched controls and to unaffected female relatives of children with CAH, and in studies using questionnaires, interviews and behavioral observation (Hines, 2011b, 2015). Females with CAH also have been found to show reduced female gender identity (Pasterski et al., 2015; however, see Meyer-Bahlburg et al., 2004) and reduced heterosexual interests (Frisén et al., 2009; Hines et al., 2004; Meyer-Bahlburg et al., 2008).

Females with CAH also have been found to be more aggressive than unaffected female controls. For example, Berenbaum and Resnick (1997) studied aggression in 49 females and 41 males with CAH, ranging in age from approximately 3 to 35 years, and in their unaffected, similarly-aged relatives (28 females, 48 males). Data were analyzed separately for adult, adolescent and preadolescent participants. In all three samples, females with CAH scored higher on aggression than unaffected females, although the difference was not statistically significant in the preadolescent sample. Similarly, Pasterski et al. (2007) compared 3- to 11-year-old children with CAH (38 girls, 29 boys) to their unaffected siblings (25 girls, 21 boys), and found that girls with CAH were rated by their mothers as being more aggressive than unaffected girls. Finally, Mathews et al. (2009) studied recalled aggressive behavior at age 12 to 13 years in individuals with CAH (40 females, 29 males), aged 12 to 45 years, and their unaffected, similarly-aged relatives (29 females, 30 males), and found that females with CAH reported greater physical aggression than unaffected females.

Studies of activity level in children with CAH have generally found that girls with CAH show higher levels of activity than unaffected girls. For example, Ehrhardt et al. (1968) assessed physical energy expenditure in 15 girls with CAH, aged between 5 and 16 years, and 15 matched controls. Eleven of the girls with CAH were reported (by mothers or the children themselves) to engage in intense outdoor activities compared to only five of the matched controls. Similarly, Ehrhardt and Baker (1974) compared 17 girls and young women with CAH, aged 4 to 19 years, to 11 unaffected female siblings, aged 6 to 24 years. More females with CAH than unaffected females were described by their mothers as having a high level of intense physical energy expenditure. Finally, Pasterski et al. (2007) compared 3- to 11-year-old children with CAH (38 girls, 29 boys) to unaffected relatives of similar age (25 girls, 21 boys), and found that girls with CAH were rated by their mothers as being more active than unaffected girls.

Another approach to assessing influences of prenatal testosterone exposure on human behavior has involved measuring testosterone concentrations in typically-developing individuals. For example, testosterone concentrations have been measured in amniotic fluid obtained during clinical amniocentesis (Cohen-Bendahan et al., 2005; Constantinescu and Hines, 2012). Testosterone enters the amniotic fluid via diffusion through the fetal skin during early gestation and, from mid-gestation onwards, through fetal urination and lung fluid secretion (Brace, 1997; Robinson et al., 1977). The timing of amniocentesis, which is typically performed during the second trimester of pregnancy, coincides with the period during gestation when the sex difference in fetal testosterone exposure is large, between 8 and 24 weeks' gestation (Smail et al., 1981). Thus, amniocentesis may provide a means for accessing a key developmental period during which testosterone influences human sexual differentiation.

Researchers have reported significant relations between concentrations of testosterone measured in amniotic fluid and some behaviors that differ on average for males and females, including empathy (Chapman et al., 2006) and traits related to autism (Auyeung et al., 2009b; Auyeung et al., 2006; but, see Kung et al., 2016). There have been no studies to date, however, relating amniotic fluid testosterone to either aggression or activity level.

We conducted two studies to investigate the hypothesis that prenatal androgen exposure, in both the typical and atypical range, predicts increased aggression and activity. In the first study (the “CAH study”), we assessed aggression and activity level in children exposed to unusually high concentrations of adrenal androgens prenatally because of CAH and in their unaffected relatives, but using a larger sample than has been used in most prior studies. In the second study (the “amniotic testosterone study”), we assessed aggression and activity level in typically-developing children for whom testosterone had been measured in amniotic fluid. We evaluated three specific hypotheses: (1) typically-developing boys show higher levels of aggression and activity than typically-developing girls; (2) girls with CAH show higher levels of aggression and activity than girls without CAH; and (3) concentrations of amniotic fluid testosterone relate positively to aggression and activity level in boys and in girls.