Fetal Testosterone Predicts Sexually Differentiated Childhood Behavior in Girls and in Boys

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/rhr/Bonnie Auyeung et al.

/sh/*Research Report*

/a/Fetal Testosterone Predicts Sexually Differentiated Childhood Behavior in Girls and in Boys

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/ab/**ABSTRACT**—Mammals, including humans, show sex differences in juvenile play behavior. In rodents and non-human primates these behavioral sex differences result, in part, from sex differences in androgens during early development. Girls exposed to high levels of androgen prenatally, because of the genetic disorder, congenital adrenal hyperplasia, show increased male-typical play, suggesting similar hormonal influences on human development, at least in females. We report here that fetal testosterone measured from amniotic fluid relates positively to male-typical scores on a standardized questionnaire measure of sex-typical play in both boys and girls. These results show, for the first time, a link between fetal testosterone and the development of sex-typical play in children from the general population, and are the first data linking high levels of prenatal testosterone to increased male-typical play behavior in boys.

/text/Sexual differentiation of the mammalian brain occurs under the control of gonadal hormones, particularly androgens, during early development (De Vries & Simerly, 2002; Ehrhardt & Meyer-Bahlburg, 1981; Goy & McEwen, 1980). Manipulating androgens prenatally or neonatally permanently alters brain regions and behaviors that show sex differences (De Vries & Simerly, 2002; Goy & McEwen, 1980; Hines, 2004). For instance, in rodents and non-human primates, treating developing females with testosterone or other androgens increases male-typical

play, whereas reducing androgens in developing males reduces it (Goy & McEwen, 1980; Hines, 2004). Androgen exerts similar effects on sex-typed reproductive behaviors and on neural sex differences (De Vries & Simerly, 2002).

In humans, sex differences in toy preferences have been observed in children as young as 12 months of age (Servin, Bohlin, & Berlin, 1999; Snow, Jacklin, & Maccoby, 1983), and these differences, along with sex differences in playmate and activity preferences, grow larger as children progress into middle childhood (Golombok & Hines, 2002). The strongest evidence that androgens influence human sexual differentiation comes from studies of play behavior in girls exposed to abnormally high levels of androgens because of congenital adrenal hyperplasia (CAH), a genetic disorder that causes excess adrenal androgen production beginning prenatally (New, 1998). Several research groups have reported that girls with CAH show increased male-typical toy, playmate, and activity preferences (Ehrhardt & Meyer-Bahlburg, 1981; Hines, 2003, 2004; Pasterski et al., 2005). Because girls with CAH are treated postnatally to normalize hormones, this behavioral masculinization is thought to result from prenatal androgen exposure. However, CAH-related disease characteristics, rather than prenatal androgen exposure, could be responsible (Fausto-Sterling, 1992; Quadagno, Briscoe, & Quadagno, 1977).

Studies relating prenatal testosterone to play behavior in typically-developing children have produced mixed results. One study, based on a large population sample, reported a positive relationship between maternal testosterone during pregnancy and male-typical play in girls, but not boys

(Hines, Golombok, Rust, Johnston, & Golding, 2002). However, it has been suggested that these results could reflect mothers with high testosterone encouraging more male-typical play in their daughters, rather than an effect of testosterone on the developing brain (Cohen-Bendahan, van de Beek, & Berenbaum, 2005). Also, two studies have found no relationship between testosterone measured in amniotic fluid and subsequent sex-typical play (Knickmeyer et al., 2005; van de Beek, van Goozen, Buitelaar, & Cohen-Kettenis, 2008). The first study found no relationship between testosterone and maternal reports of childhood sex-typed activities for 22 girls and 31 boys, and the second found no relationship between testosterone and observed toy choices in 63 girls and 63 boys. These negative results could reflect small samples or insufficiently sensitive behavioral measures.

Direct measurement of fetal testosterone and of childhood sex-typed behavior in a large sample of girls and boys, using a sensitive, reliable, standardized measure could clarify the role of testosterone in human sexual differentiation. In the human fetus, testosterone enters the amniotic fluid via diffusion through the fetal skin, and later via fetal urination (Robinson, Judd, Young, Jones, & Yen, 1977). Testosterone measured in amniotic fluid shows variability in both sexes, but is higher on average in male than in female fetuses (Martin, 1985). In the present study, fetal testosterone was measured in amniotic fluid from 212 pregnant women and related to subsequent sex-typed behavior assessed using a standardized measure, designed specifically to detect differences in sex-typed behavior within each sex.

/h1/**METHOD**

/h2/**Participants**

/text/Participants were recruited from a longitudinal study of the effects of fetal testosterone on child development. All mothers had undergone routine amniocentesis in the Cambridge, United Kingdom, region and given birth to healthy singleton infants (Baron-Cohen, Lutchmaya, & Knickmeyer, 2004). Materials for the present study were sent to all 452 available mothers, who were asked to complete a questionnaire about their child's activities and interests. Complete information was obtained for 112 male and 100 female offspring (mean age = 8.59 years, *SD* = 0.97 years, range = 6.38–10.30 years). No significant differences were observed for the predictor or control variables between the larger and current sample in this study.

/h2/**Measures**

/h3/*Outcome Variable*

/text/The Preschool Activities Inventory (PSAI) is a psychometric scale, with established validity and reliability, developed specifically to assess variability in sex-typical behavior within each sex (Golombok & Rust, 1993a, 1993b). It includes 24 items and is completed by a parent to describe the child's behavior. Higher scores reflect more male-typical behavior, and females with CAH obtain elevated (more male-typical) scores on the PSAI in comparison to unaffected female relatives (Hines, Brook, & Conway, 2004), suggesting sensitivity to the effects of prenatal androgen exposure.

/h3/*Predictor Variable*

Amniotic fluid samples were collected between weeks 11 and 21 of gestation (M = 16.31, SD = 1.88). This timing coincides with the hypothesized critical period for human sexual differentiation, which is thought to occur between approximately weeks 8 and 24 of gestation (Hines, 2004). Fetal testosterone was measured in amniotic fluid via radioimmunoassay with ether extraction using the DPC "Count a Coat" method (Diagnostic Products Corporation, Los Angeles, CA), which uses an antibody to testosterone coated onto propylene tubes and a 125-I labelled testosterone analogue. The detection limit of the assay using the ether-extraction method is approximately 0.1 nmol/L. The coefficient of variation (CV) for between batch imprecision is 19% at a concentration of 0.8 nmol/L and 9.5% at a concentration of 7.3 nmol/L. The CV's for

within-batch imprecision are 15% at a concentration of 0.3 nmol/L and 5.9% at a concentration of 2.5 nmol/L. This method measures total extractable testosterone.

/h3/*Control Variables*

/text/Gestational age at amniocentesis, maternal age, maternal education, and child's age at PSAI assessment were included for control purposes. Gestational age and child's age were assessed from medical records. Maternal age and education were assessed by self-report, with education rated on a 5-point scale from 1 (*no formal qualifications*) to 5 (*postgraduate qualification*).

/h1/**RESULTS**

/text/There were two female outlier values for fetal testosterone, but no male outliers (see Fig. 1). Because of the outliers, analyses involving testosterone in females were first conducted by using the full data set and then repeated excluding the outliers. No outliers were observed for PSAI scores or control variables. All variables had acceptable skewness statistics (<1.0).

As expected, boys had higher amniotic testosterone than girls, boys: $M = 0.83$, $SD = 0.43$; girls: $M = 0.33$, $SD = 0.32$; $t(202.66)=9.67$, $p < .001$, unequal variance; with the female outliers removed, girls: $M = 0.29$, $SD = 0.20$; $t(161.58) = 11.71$, $p < .001$, unequal variance, and higher PSAI scores, boys: $M = 68.95$, $SD = 10.73$; girls: $M = 34.95$, $SD = 12.48$; $t(210)=21.32$, $p < .001$. None of the control variables showed a sex difference (see Table 1 for descriptive statistics and correlations of PSAI scores with fetal testosterone and control variables).

Data were first analyzed for both sexes combined by using backward stepwise linear regression using all fetal testosterone values. Any variable that correlated with PSAI scores at $p < .2$ was entered into the analysis (Altman, 1991). In addition, the influence of suppressor variables (variables that correlate highly with other predictors in the model, $p < .01$) was investigated. Maternal education, maternal age, child's age, fetal testosterone levels, sex, and the interaction between sex and fetal testosterone were included in the analysis (entry criterion $p < .05$, removal criterion, $p > .10$). The only significant predictors included in the final model were sex and fetal testosterone, $R^2 = .73$, $F = 250.11$, $p < .001$. The results remained the same when the two female outliers were excluded from the analysis, and the only predictors retained in the final model were sex and fetal testosterone, $R^2 = .73$, $F = 248.63$, $p < .001$.

Within-sex analyses on the full data set indicated that testosterone correlated positively with PSAI scores for both girls ($r = .42$, $p < .001$) and boys ($r = .20$, $p < .05$). The size of the correlations between PSAI scores and fetal testosterone level did not differ significantly between the

sexes ($z = 1.75$, $p > .05$) (see Fig. 1 and Tables 1 and 2). Without the two female outliers, the correlation for girls was $r = .33$, $p < .001$. For within-sex regression analyses, the same predictor variable selection procedure described above was used. For girls, maternal age, child's age (suppressor), and fetal testosterone levels were included in the analysis. Fetal testosterone level was the only significant predictor retained in the final model, $R^2 = .11$, $F = 5.53$, $p < .01$. In the regression analysis without the two female outliers, fetal testosterone level was the only variable retained in the final model $R^2 = .09$, $F = 8.76$, $p < .01$. For boys, fetal testosterone level and maternal age were entered in the analysis. The final regression model for boys retained both fetal testosterone level and maternal age, $R^2 = .08$, $F = 4.36$, $p < .05$.

/h1/**DISCUSSION**

/text/We found a significant relationship between fetal testosterone and sexually differentiated play behavior in both girls and boys. The large sample, focus on middle-childhood when sex differences in play are strongest and the specific measure used may account for our ability to detect this relationship, even though two prior studies did not detect the relationship (Knickmeyer et al., 2005; van de Beek et al., 2008).

Because children in the current study were developing typically, and because measures of testosterone were taken directly from the fetal environment, our results strengthen the evidence that testosterone plays a role in sexual differentiation of human behavior. Prior studies linking

prenatal testosterone to childhood play have relied on clinical populations or measures of maternal hormone levels. Our study avoids problems of interpretation associated with those approaches.

Our results also differ from prior findings in that we found a relationship between prenatal testosterone and sex-typical play in both boys and girls. In contrast, studies of children with CAH have reported elevated male-typical behavior in girls but not boys (Hines, 2004), and the prior study relating maternal testosterone during pregnancy to childhood behavior found a relationship in girls but not boys (Hines et al., 2002). Thus, our data are the first documentation that androgen exposure prenatally relates to sexually differentiated play behavior in boys and in girls. In addition, the current results support an organizational, as opposed to current, activational role of testosterone, because play behavior is measured in childhood, when concurrent testosterone levels are low.

Prior difficulty finding predicted relationships between testosterone and behavior in boys may reflect the use of approaches that are not well-suited to its detection. Boys with CAH, unlike girls, may adjust testicular androgen production prenatally, thus avoiding marked androgen elevation (Hines, 2003), and testosterone in mothers and daughters correlate, whereas there is no correlation between testosterone in mothers and sons (Harris, Vernon, & Boomsma, 1998), making maternal samples less useful for detecting hormone-behavior relationships in boys. Therefore,

studies relating amniotic fluid testosterone to subsequent behavior may be particularly useful for elucidating the role of testosterone in the behavioral development of boys.

/ack/***Acknowledgments***—This work was supported by grants from the Nancy Lurie-Marks Family Foundation and the Medical Research Council (to S.B.C.) and by National Institutes of Health Grant HD 24542 (to M.H.). B.A. was supported by a scholarship from Trinity College, Cambridge. We are grateful to the families who have taken part in this longitudinal study over many years and to Ian Goodyer, Greg Davis, and Ieuan Hughes for valuable discussions.

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/ra/(Received 4/3/08; Revision accepted 7/28/08)

/tc/TABLE 1

Descriptive Information and Correlations with Preschool Activities Inventory (PSAI) Scores[Au: What is correlated with PSAI scores?]

	Combined group					Girls					Boys				
Variable	*n*	*M*	*SD*	Range	*r*	*n*	*M*	*SD*	Range	*r*	*n*	*M*	*SD*	Range	*r*
Fetal testosterone level (nmol/L)	212	0.59	0.46	0.05–2.30	0.59**	100	0.33	0.32	0.05–2.30	0.42*	112	0.83	0.43	0.05–2.05	0.20*

Gestational age (weeks)	145	16.31	1.88	11.00–21.50	−0.01	67	16.31	2.32	11.0–19.0	−0.04	78	16.32	1.5	13.0–20.0	0.01
Child's age (years)	209	8.59	0.97	6.35–10.29	0.11	99	8.44	1.02	6.35–10.27	−0.01	110	8.70	0.93	6.57–10.29	−0.06
Maternal age (years)	191	35.11	8.02	22.81–46.42	−0.10	91	35.31	4.10	22.81–46.42	0.16	100	34.90	4.78	23.38–44.14	−0.20*
Maternal education level	186	3.11	1.06	1–5	0.21**	88	2.88	0.90	2–5	0.08	98	3.21	1.25	1–5	0.04
PSAI score	212	52.91	20.57	13–86	1.00	100	34.95	12.48	13–78	1.00	112	68.95	10.73	24–86	1.00

/tfn/Note. The table presents raw values for fetal testosterone levels. Maternal education level was self-reported and rated on a 5-point scale from 1 (*no formal qualifications*) to 5 (*postgraduate qualification*).

*$p < 0.05$. **$p < 0.01$

/tc/TABLE 2
Final Hierarchical Regression Model for the Preschool Activities Inventory

Group and predictor	R^2	β	*SE*	*p*
Combined	.73			
Fetal testosterone level		.14	2.28	<.01
Sex		.77	1.94	<.001
Girls	.11			
Fetal testosterone level		.27	4.17	<.05
Maternal age		.18	0.09	>.05
Boys	.04			
Fetal testosterone level		.21	2.66	<.05
Maternal age		.20	0.12	<.05

[Au: Please clarify whether the table shows one model or three (one for each group)]