Neonatal Sex-Steroid Hormones and Cognitive Abilities at Six Years

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Five sex-steroid hormones (testosterone, androstenedione, estradiol, estrone, and progesterone) were assayed in umbilical cord blood. Cognitive abilities were assessed as a part of a 6-year follow-up laboratory visit. Four subtests were given: reading, numbers, listening, and spatial ability. There were no significant differences between boys and girls in cognitive ability scores. Higher levels of perinatal androgens (testosterone and androstenedione) were significantly associated with low age-6 spatial ability in girls. Multiple regression analyses revealed a significant proportion of the variance in cognitive abilities in girls could be accounted for by testosterone and androstenedione. No significant predictions were found for boys. The finding of a stable inverse association between sex and effect of hormones on abilities is discussed.

Whether sex-steroid hormones are associated with cognitive abilities in boys and girls has been an intriguing question. There are both sex-related differences in cognitive abilities and in circulating sex-steroid hormones beginning in adolescence. However, when one tries to find a causal link, either in associations between hormonal measures and concurrent cognitive abilities or early hormonal and later cognitive abilities, the relationship is not clear.

Hormones may have the greatest effect early in fetal development when levels of the sex-steroid hormones are quite high (Faiman, Reyes, & Winter, 1974). The theoretical argument is that these sex-steroid hormones change the fetal brain even though behavioral effects may not be seen for years. The

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theory is better instantiated with animal than human studies. (See Hines, 1982, for a review.)

Several studies have tried to assess the effects of prenatal hormones and later intellectual behaviors in humans, but the picture is incomplete. With populations of fetally androgenized girls, Ehrhardt and Money (1967) reported higher intelligence test scores than would have been expected for the general population. However, it was later found that although fetally androgenized girls did have higher-than-average IQ scores, so too did their sisters who had not been fetally androgenized (Erhardt & Baker, 1973). Similar findings are reviewed by Ehrhardt and Meyer-Bahlburg (1979). The Ehrhardt and Money findings, then, might well have reflected a selection of subjects from more advantaged segments of the population. However, a recent study of females with congenital adrenal hyperplasia (CAH), an autosomal recessive disorder associated with elevated adrenal androgen, found that CAH females, as compared with unaffected female relatives, showed significantly enhanced performance on three tests of spatial ability (Resnick, Berenbaum, & Gottesman, 1986).

Other investigations of prenatal exposure to hormones find relationships between the exposure and later development. In one study, both boys and girls who received large dosages of progesterone in utero, had higher general aptitude than matched controls at seven years of age, as reported by their teachers (Dalton, 1968). These children are more likely to enter University, and they leave school with higher grades than their non-progesterone-treated controls (Dalton, 1976). However, other work has not replicated this finding with lower dosages of progesterone (Meyer-Bahlburg & Ehrhardt, 1977).

No difference in intelligence test results were found in groups prenatally treated with estrogen, progestins or combinations compared to untreated sibling controls (Reinisch & Karrow, 1977). Women given the synthetic estrogen, diethylstilbestrol (DES) prenatally did not differ from their nontreated sisters in cognitive test scores (Hines, 1984). Similarly, men given estrogens prenatally did not have different cognitive test scores than matched controls (Kester, Green, Finch, & Williams, 1980).

Unfortunately, in addition to being equivocal, the majority of the work cited above on prenatal hormone associations with later cognitive ability has been with exogenous hormones. That is, for therapeutic reasons, hormones were administered to the pregnant mother and the children's abilities were later tested. These exogenous hormones differ in quantity and quality from the circulating hormones of the placenta and fetus. Work has not thus far been reported for endogenous hormones in a normal population.

The present study addresses the following issues: (1) Are there sex differences in cognitive abilities at 6 years of age?; and (2) are cognitive abilities at 6 years of age related to nenonatal sex-steroid hormones in boys and girls in a normal population.

Method

Sample

The sample of children were a part of the Stanford Longitudinal Study. Ninety-six of the children, who were initially seen by the experimenters at birth, were recontacted and tested at 6 years of age as a part of a longitudinal follow-up. Cohort I, II, and III, from the original study, will be combined for the present

paper. Of the original cohorts, cohort I and II comprised all the children born in a university hospital and a general hospital during the summer of 1973 and winter and spring of 1974, and cohort III was comprised of the children born in the general hospital in the fall of 1975, who met the specific criteria, viz: (1) no complications of pregnancy or delivery, (2) a 5-min Apgar score of 7 and above, (3) 15 cubic centimeters (cc) of umbilical cord blood taken at birth (by the delivery room nurses), and (4) permission obtained from the parents for the family to participate in the longitudinal study.

The birth hormone measures of all of the children in the original sample does not differ from the smaller sample that was recontacted and tested at 6 yr of age.

Procedure

Hormones

At the time of each infant's birth, at least 15 cc. of blood were taken from the umbilical cord as soon as it was severed. The blood was predominantly venous, with relatively smaller amounts from the cord artery. The blood was allowed to clot in a refrigerator and the serum was stored frozen at −25°F until assay. On the day following the infant's birth, the mother was asked for permission to include the infant in the longitudinal study. For the infants so enrolled (a large majority of those eligible) the frozen serum samples were subsequently analyzed for five hormones. Androstenedione (4-androsten-3, 17-dione), testosterone (17B-hydroxy-4-androsten-3-one), estrone (3-hydroxy-1,3,5(10)-estratrien-17-one), and estradiol (1,3,5(10)-estratriene-3, 17B-diol) were estimated by radioimmunoassay methods. Progesterone (4-pregnen-3, 20-dione) was assayed by a competitive protein binding method.

It should be noted that the birth concentrations of testosterone were greater in males than females. The other four hormones did not differ significantly by sex. Further details of the assays and hormone relationships are described in Maccoby, Doering, Jacklin, and Kraemer (1979).

Cognitive Testing

At age $6\frac{1}{2}$, each child was given a cognitive test by a trained experimenter as a part of the 6-yr laboratory visit. The cognitive testing was done after an initial warm-up game played by the parents and child. The test consisted of four subtests. The first three subtests (reading, numbers and listening were taken from the kindergarten and first grade versions of the Metropolitan Instructional Tests (1979). The fourth subtest, spatial ability, was taken from the kindergarten and first grade versions of the Primary Mental Abilities Test.

The scores of the subtests were positively correlated in both boys and girls, thus summary scores were constructed. The first summary score or Total Academic Cognitive Score is a summed score of the four subtests. In addition, a principal components analysis was done of the four subtests, with two components emerging. The first principal component was a general academic cognitive cluster accounting for .585 proportion of the variance. This is called the General Academic Component. A second principal component was a spatial cluster and it accounted for .180 proportion of the variance. This is referred to as the Spatial Component.

TABLE 1. Cognitive Ability Scores for Boys and Girls at 6½ Years.

	Boys (n = 53)	Girls (n = 43)	t	Two-Tail Probability
TAC	396.58	404.85	−1.35	.18
GAC	−.18	.23	−1.33	.19
SC	−.03	.04	−0.38	.70

* TAC = Total Academic Cognitive Score, GAC = General Academic Component, SC = Spatial Component.

Results

Cognitive scores for boys and girls are presented in Table 1. There are no significant differences in the Total Academic Cognitive Score, General Academic Component, or Spatial Component.

The strength of association between the five sex-steroid hormones assayed at birth and the cognitive measures was examined using a Pearson correlation coefficient. Results are presented in Table 2. The male hormones, testosterone and androstenedione, are negatively and significantly associated with the Spatial Component in girls ($r = -.34$ & $r = -.37$; $p = .05$). No other significant associations are seen. A consistent pattern of opposite hormone/cognitive associations for boys and girls is found with the hormones testosterone, estradiol, and progesterone and the Total Academic Cognitive Score and General Academic Component. All correlations are in the negative direction for boys, while for girls all correlations are in the positive direction.

The neonatal hormone scores are intercorrelated. In psychological correlational research on individual differences, a common method of dealing with cor-

TABLE 2. Correlations of Hormones and Cognitive Ability Scores.

	TAC	GAC	SC
		Testosterone	
Boys	−.028	−.025	−.085
Girls	.264	.264	−.337*
		Androstenedione	
Boys	−.190	−.184	−.190
Girls	−.260	−.251	−.366*
		Estradiol	
Boys	−.152	−.145	−.236
Girls	.019	.031	−.152
		Estrone	
Boys	−.267	−.262	−.260
Girls	−.138	−.137	−.149
		Progesterone	
Boys	−.117	−.119	−.151
Girls	.005	.001	−.072

* $p = .05$. TAC = Total Academic Cognitive Score, GAC = General Academic Component, SC = Spatial Component.

relations among predictive variables is to identify the component that they have in common, determine the effect of this component, and then determine whether the individual variables have any predictive power that is independent of the common component. Principal component analysis was used to identify the shared component of the five sex-steroid hormones (see Marcus, Maccoby, & Jacklin, 1985, for further details of the hormone analysis). The analysis revealed a primary component that accounted for 59% of the variance in hormone levels and is called the "all hormone" component. All hormones had substantial and significant loadings, of about equal magnitude, on this component. A second component, accounting for 18% of the variance, had positive loadings on androstenedione and testosterone, and a negative loading on progesterone. The second component is labeled the "androgen" component. Two hormone component scores were derived for each child by weighing each individual's hormone scores by the loading of each hormone in the principal component analysis. Sex differences were found in the two hormone component scores. The mean scores for male neonates is significantly higher than for females on both components ($p = .01$ "all hormone" component; and $p = .001$ "androgen" component).

First-order correlations between the hormone component scores and the cognitive ability scores are reported in Table 3. There is no significant relationship between the Total Academic Cognitive Score or General Academic factor and the hormone components. There is a significant negative association between the Spatial factor in girls and the androgen component score ($p = .01$).

Stepwise multiple regression analyses were performed for all hormone scores and all cognitive ability measures to examine to what extent particular neonatal sex-steroid hormones could predict cognitive ability in boys and girls at 6 years of age. For boys, estrone and estradiol accounted for approximately 10% of the variance in the Total Academic Cognitive Score, General Academic Component, and Spatial Component. F tests on the coefficient of determination (r squared) were nonsignificant for all cognitive ability measures. The contributions of progesterone, testosterone, and androstenedione were negligible to any prediction of cognitive ability measures in boys.

For girls, approximately 20% of the variance in Total Academic Cognitive Score, General Academic Component, and Spatial Component, was accounted for by testosterone and androstenedione. F tests for all three cognitive measures were significant with $p = .025$. Progesterone contributed slightly to the prediction,

TABLE 3. Correlations of Hormone Component Scores and Cognitive Ability Scores by Sex.

	All Hormone Component		Androgen Component	
	Boys	Girls	Boys	Girls
TAC	−.207	−.073	.027	.032
GAC	−.202	−.072	.030	.040
SC	−.245	−.256	.029	−.398**

** $p = .01$. TAC = Total Academic Cognitive Score, GAC = General Academic Component, SC = Spatial Component.

with estrone and estradiol having a negligible effect. A multiple regression with sex, androgen component score and the interaction of sex × androgen component as predictors of the Spatial Factor was performed to test for significant interactions between these variables. The test was nonsignificant at the $p = .05$ level.

Discussion

In this study, no significant differences were found in cognitive abilities between males and females tested at 6 years. However, boys and girls show systematic differences in the pattern of relationships between their 6 years abilities and their neonatal sex-steroid hormones. For three of the hormones: testosterone, estradiol, and progesterone, correlations were in opposite directions for boys and girls. These findings echo previous findings between hormones and behaviors found by Jacklin, Maccoby, and Doering, (1983) and Jacklin, Maccoby, Doering, and King (1984). In examining the relationships between hormones and strength, and hormones and timidity in boys and girls the following different directions in associations were found. In boys, neonatal progesterone levels were positively related to strength measures and inversely related to timidity; whereas in girls, progesterone was negatively related to strength and unrelated to timidity. In the present study, there was a significant relationship between male hormones and spatial abilities in girls. An inverse association between sex and effect of hormones seems to exist.

While systematic associations of direction were found between hormones and cognitive abilities for boys and girls, the correlations were only significant for testosterone and androstenedione and spatial abilities in girls. These findings are contrary to previous research on hormones and spatial abilities which found a positive relationship between androgens and spatial abilities in females (Resnick et al., 1986). In this study, a negative association was found.

These inconsistencies in the literature lead to the larger question of how to study hormone-behavior relationships in humans. One approach has been to study "atypical" populations, such as the effect of prenatal exposure to exogenous hormones used for the treatment of at-risk pregnancies (Hines & Shipley, 1984) or individuals with autosomal disorder, such as CAH. These "experiments in nature" are important in human subjects where other experimentation is impossible. However, an obvious problem with this research is that the findings concern atypical populations and may not be generalizable to normal populations. A methodology that utilized a "normal" population entails taking samples of cord blood from infants at the time of delivery, such as was done in the present study. Sex hormone levels are then ascertained from the cord blood samples. While this method circumvents the problem of using an atypical sample, it is not without other problems.

First, it is not clear how the onset of labor and the stress of labor and delivery affect the levels of hormones found in cord blood (Fuchs & Fuchs, 1984). Second, the hormones present at labor and delivery may not be representative of the hormone levels present in the fetus during the time of maximum sensitivity for brain exposure to hormones. In humans, this time of maximum sensitivity has not been specified, although, it is believed to be in early or midgestation (Hines, 1982; Reinsich & Sanders, 1984). Further clarification of the sensitizing effects of endogenous hormones in normal humans may have to wait until safe methods are devised to measure sex-steroid hormones in the fetus during the midgestational period.

The repeated findings of differential effects of sex-steroids on males and females is curious. This has been reported in our work with very young children and recently with concurrent hormones in older children (Susman, Inoff-Germain, Nottelman, Loriaux, Cutler, & Chrousos, 1987). Sometimes effects are only found in one sex and sometimes opposite effects are found for the same hormone in boys and girls. We cannot explain this phenomenon at present. The explanation may have to await understanding the mechanism linking hormones and brain organization (Crockett & Petersen, 1984).

However, one plausible hypothesis is that the ranges of hormone sensitivity may be different for the sexes. That is, what is too much or too little hormone may be different for the developing girl or boy. A given amount of progesterone then, for example, could effect the sexes differently as has been demonstrated (Jacklin et al., 1984). Or, as in the present study, having a given amount of male hormone may be deleterious to females, but not affect males in their behavior at all. Looking at the actual range of hormone scores is one mechanism that could serve to separate the endogenous hormones studies from exogenous hormone studies. Unfortunately, exogenous hormones differ at present from endogenous hormones in both quantity and quality.

Additional studies need to be carried out with perinatal endogenous hormone samples in order to understand the complex picture of the hormone-behavior phenomenon. Hopefully, it will soon also be possible to safely study prenatal endogenous hormones. While research with both normal and atypical populations support the hypothesis that differential hormone level or exposure influences later patterns of cognitive behavior, it is not yet clear what direction this contribution may take and how large an influence hormones have on later behavior.

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