Hormone–behavior associations in early infancy

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ABSTRACT

The physiological significance of hormonal changes in early postnatal life is emerging, but the behavioral significance in humans is unknown. As a first test of the relationship between hormones and behavior in early infancy we measured digit ratios and salivary hormone levels in forty-one male and female infants (3–4 months of age) who watched a video depicting stimuli differentially preferred by older males and females (toys, groups). An eye-tracker measured visual fixations and looking times. In female infants, hormones were unrelated to visual preferences. In male infants, higher androgen levels predicted stronger preferences for male-typical stimuli. These data provide the first evidence for a role for hormones in emerging sex-linked behavior in early development.

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In humans, the activation of the hypothalamic-pituitary-gonadal (HPG) axis contributes to the well-recognized physical and behavioral correlates of gender. In prenatal life, the increased testicular production of androgens masculinizes and defeminizes the genitalia, as well as the developing neural system (Breedlove et al., 1999; Hines, 2004). Further, converging evidence from research on clinical and nonclinical populations suggests that this organizing influence of androgens on the developing brain also contributes to the greater expression of male-typical behavior (Collaer and Hines, 1995). With puberty, an increased production of sex steroids acting on tissue organized in early development results in the expression of secondary sex characteristics (e.g., breast development in females, facial hair in males) and establishes fertility (Sisk and Foster, 2004). Unlike the early, permanent effects of hormones on the developing body, these postnatal hormone effects may vary according to the availability of hormone. For example, it is well established that inhibition of the HPG axis can suppress both fertility and male sexual motivation (Davidson et al., 1979).

Largely absent from this classic understanding of the role of hormones in the development of sex differences in human behavior is the function and significance of the surge in reproductive steroids that results in early postnatal life from a transient activation of the HPG axis. In infant boys, serum testosterone levels increase to peak levels around 3 months of age and fall to prepubertal values around 6 months of age (Forest et al., 1974). In infant girls, a similar increase occurs in serum estradiol—although with much greater interindividual variability (Chellakooty et al., 2005). Findings from salivary measures suggest that the levels of biologically active hormone are much lower than those in adulthood (Huhtaniemi et al., 1986), results that are consistent with more recent research showing free

testosterone levels in neonates are well below the levels of free testosterone in adult men (de Ronde et al., 2005). Nonetheless, evidence of the physical significance of postnatal hormone levels is emerging. For example, the postnatal increase in testosterone levels is critical for the normal development of male genitalia and reproductive function (Main et al., 2005) and breast tissue response to levels of estradiol in female infants is thought to contribute to breast development in adulthood (Schmidt et al., 2002). From the perspective of classic hormone–behavior theory, this sex-specific sensitivity to the presence of hormone in early infancy and the subsequent effects on physical development suggests that hormone levels in early postnatal life may also contribute to sex differences in human behavior. Yet, to date, there are no studies examining the association between hormone levels and behavior during early infancy.

Accordingly, the aim of this study was to provide a first test of the postnatal hormone hypothesis. Visual interest in toys and group stimuli (i.e., multiple individuals) was measured as emerging sex-linked behavior because: (1) older children show well-established sex differences in toy preferences and social organization, such that boys show stronger preferences for toys such as balls, vehicles, and construction toys (Alexander and Hines, 1994) and for social interactions with larger numbers of individuals (Benenson et al., 2007); and (2) older infants show sex differences in visual preferences for toys (Alexander et al., 2009) and groups (Benenson et al., 2004). Finally, similar sex differences in behavior have been reported in other primates (Alexander and Hines, 2002; Hassett et al., 2008), suggesting that a biological predisposition for the preferential visual processing in infancy may contribute to the later sex differences in human behavior (Alexander, 2003; Benenson et al., 2007). We examined the association between developing sex differences and androgens by measuring eye movements using eye-tracking technology and measuring levels of testosterone and estradiol in saliva. As previous research has demonstrated a role for prenatal hormones in

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Table 1
Descriptive statistics of hormone levels, digit ratios, and visual interest measures in male and female infants (mean, SD).

	Males ($n=21$)	Females ($n=20$)	Effect Size (d)
Testosterone (pg/ml)	40.68 (10.69)	38.52 (12.86)	0.18
Estradiol (pg/ml)	4.53 (0.93)	4.73 (0.86)	0.22
2D:4D ratio	0.915 (0.034)	0.926 (0.039)	0.30
Fixation (n)			
Group	3.76 (3.23)	5.48 (5.15)	0.40
Individual	1.14 (1.42)	1.95 (2.54)	0.39
Doll	2.00 (2.79)	3.89 (3.68)	0.58
Ball	1.05 (1.83)	1.89 (1.59)	0.49
Looking time (%)			
Group	6.09 (5.89)	7.11 (5.55)	0.17
Individual	1.58 (1.91)	2.86 (3.65)	0.44
Doll	2.94 (3.16)	5.37 (4.96)	0.58
Ball	1.26 (1.65)	3.46 (3.15)	0.87

the development of human sex differences (Collaer and Hines, 1995), we also measured the ratio of the second to fourth digits of the right hand, a putative marker of prenatal androgen action (McIntyre, 2006). We hypothesized that indicators of higher (i.e., more male-typical) levels of androgens as indicated by digit ratios and salivary levels of testosterone in infants would be associated with greater visual interest in a male-typical toy relative to a female-typical toy and greater visual interest in a group relative to an individual.

Methods

Participants included 21 male (M age = 3.88 months, SD = 0.64 months) and 20 female (M age = 4.04 months, SD = 0.62 months) full-term infants. An additional 3 infants were tested but excluded because of an insufficient quantity of saliva ($n=1$) or fussiness ($n=2$). The sex difference in age was small ($d=0.25$) (Cohen, 1977), and not statistically significant. Parents, who were recruited from birth announcements in the local newspaper and commercially produced lists, were offered $5 reimbursement for their travel expenses and received a small toy for their son or daughter.

Three-dimensional test stimuli (doll, ball, figures) were constructed using Autodesk Maya 6.5 software and rendered to video using Adobe AfterEffects 6.0. Each identical figure was bottle-shaped, with facial features and arms, and bounced gently. The group consisted of three figures, arranged in a semi-circle as if interacting. A video depicting the toys and figures was presented on a 20-in. computer monitor. An infra-red eye-tracker with remote optics (Model R6, Applied Science Laboratories) measured eye movements during test trials. The camera was situated directly below the computer monitor and not visible to infants. A magnetic head tracker (Flock of Birds®, Ascension Technology Corporation) was worn by infants to limit any disruption in eye-tracking as a function of head movement.

As in our previous research (Alexander et al., 2009), infants were positioned in car seats so that the camera to eye distance was approximately 56 cm. To obtain reliable and valid eye movement data, three gaze positions covering over 80% of the viewing area were first collected using small lights to direct the infants' attention to each of the three points successively. After successful calibration, each infant participated in four 5-s trials in which the stimulus pairs (group and solitary figure, ball and doll) were presented simultaneously, each pair component appearing once on the left side and once on the right side. The initial side of presentation was randomized across infants.

Saliva (<15 ml) was collected by a sterile DeLee suction catheter from each infant at the end of the session. Saliva samples were immediately stored at −80 °C. Frozen samples were shipped overnight in dry ice to Salimetrics (State College, Pennsylvania),

where salivary levels of testosterone and estradiol were measured in duplicate using enzyme immunoassays (assay sensitivity < 1 pg/ml).

The ratio of the lengths of the second and fourth digits (2D:4D) was calculated by obtaining a digital photo scan of the infant's right hand. The distance in millimeters from the basal crease to the tip of the second and fourth fingers was measured with digital vernier calipers. Two independent judges coded finger-lengths for each hand copy with excellent inter-rater reliability ($r > 0.90$).

The solitary figure, group of figures, doll and ball were each defined as an area of interest (AOI). Fixations were system defined as a period of at least 100 ms during which point of regard did not change by more than 1-degree visual angle (i.e., a distance on the display of less than 1.3 cm). Dependent variables were the number of fixations and the total looking time in each of the four AOI.

Results

Table 1 summarizes the hormonal and behavioral data in males and females. Sex differences in the testosterone, estradiol, and digit ratios were in the expected direction, but the group differences were small and not statistically significant. As shown previously (de Ronde et al., 2005; Huhtaniemi et al., 1986), the estimate of biologically active testosterone in both sexes was low compared to that in adult males, ranging from 27.51 pg/ml to 58.13 pg/ml in boys and 13.69 pg/ml to 58.72 pg/ml in girls, and did not vary significantly across the relatively narrow age range of the sample (Fig. 1). Tests of
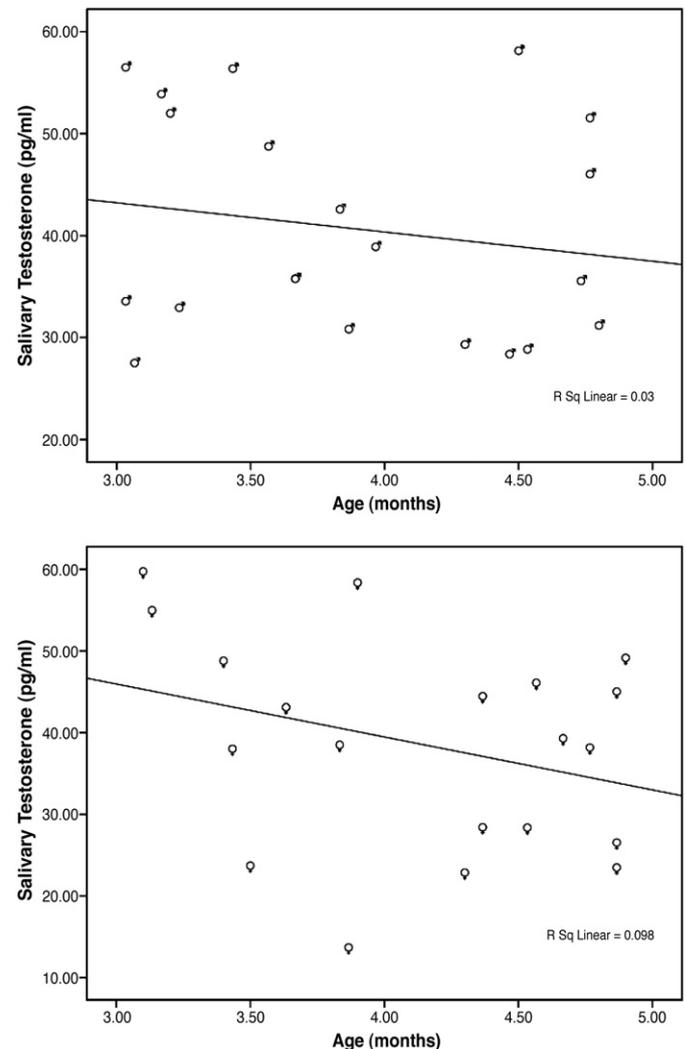

Fig. 1. Salivary testosterone levels as a function of age in male and female infants.

skewness and kurtosis indicated no significant violations of normality for the distribution of hormone levels. Visual interest in each AOI was greater in females compared to males, but the sex differences in fixation number and looking times were not statistically significant. A similar analysis using the average number of fixations and average looking times as covariates did not change these results. There were also no sex differences in difference scores calculated as measures of the relative preference for one of the two stimuli (i.e., group score− solitary figure score, ball score−doll score).

For each sex, separate hierarchical regression analyses were conducted using the preference measures (i.e., difference scores for toys and figures) as dependent variables. For each model, age and digit ratios were entered at the first step and salivary testosterone and estradiol were entered at the second step. Results of these analyses for female infants showed that the variance in the preferences for toys and groups could not be significantly accounted by any of the models or predictor variables. However, for male infants the variance in the preference for the group of figures was associated with the salivary testosterone, such that higher salivary testosterone levels predicted stronger group preferences. For fixation number, age and digit ratios accounted for a small, non-significant portion of the variance in preferences for the group, $F_{change} = 0.29$, $R^2 = 0.03$, ns. The addition of salivary hormones produced a significant $F_{change} = 6.32$, $R^2 = 0.429$, $p < 0.01$. Examination of individual beta weights indicated that salivary testosterone, $\beta = 0.676$, $p < 0.01$, contributed significantly to the variance in group preference. The results for looking time were

very similar. Age and digit ratios accounted for a small, non-significant portion of the variance in preference for the group of figures, $F_{change} = 0.09$, $R^2 = 0.0$, ns. The addition of salivary hormones produced a significant $F_{change} = 6.21$, $R^2 = 0.44$, $p < 0.01$, that was attributable to the contribution of salivary testosterone, $\beta = 0.684$, $p < 0.01$. In contrast, the variance in the preference for the ball was associated with digit ratios, such that greater prenatal androgen action (i.e., lower digit ratios) predicted stronger ball preferences. For fixation number, age and digit ratios accounted for a near significant portion of the variance in preference for the ball, $F_{change} = 3.24$, $R^2 = 0.267$, $p = 0.06$. The addition of salivary hormones did not improve the model. Examination of individual beta weights indicated that only digit ratios, $\beta = -0.434$, $p < 0.05$, contributed significantly to the variance in ball preference. The results for looking time were very similar. At the first step, age and digit ratios accounted for a significant portion of the variance in ball preference, $F_{change} = 3.63$, $R^2 = 0.287$, $p < 0.05$. The addition of salivary hormones did not improve the model. Examination of individual beta weights indicated that only digit ratios, $\beta = -0.583$, $p < 0.05$, contributed significantly to the variance in ball preference. Fig. 2 shows the significant correlations between these hormone measures and preference scores in male infants.

Discussion

This research is the first to show an association between postnatal hormone levels and sex-linked behavior in early infancy. The results
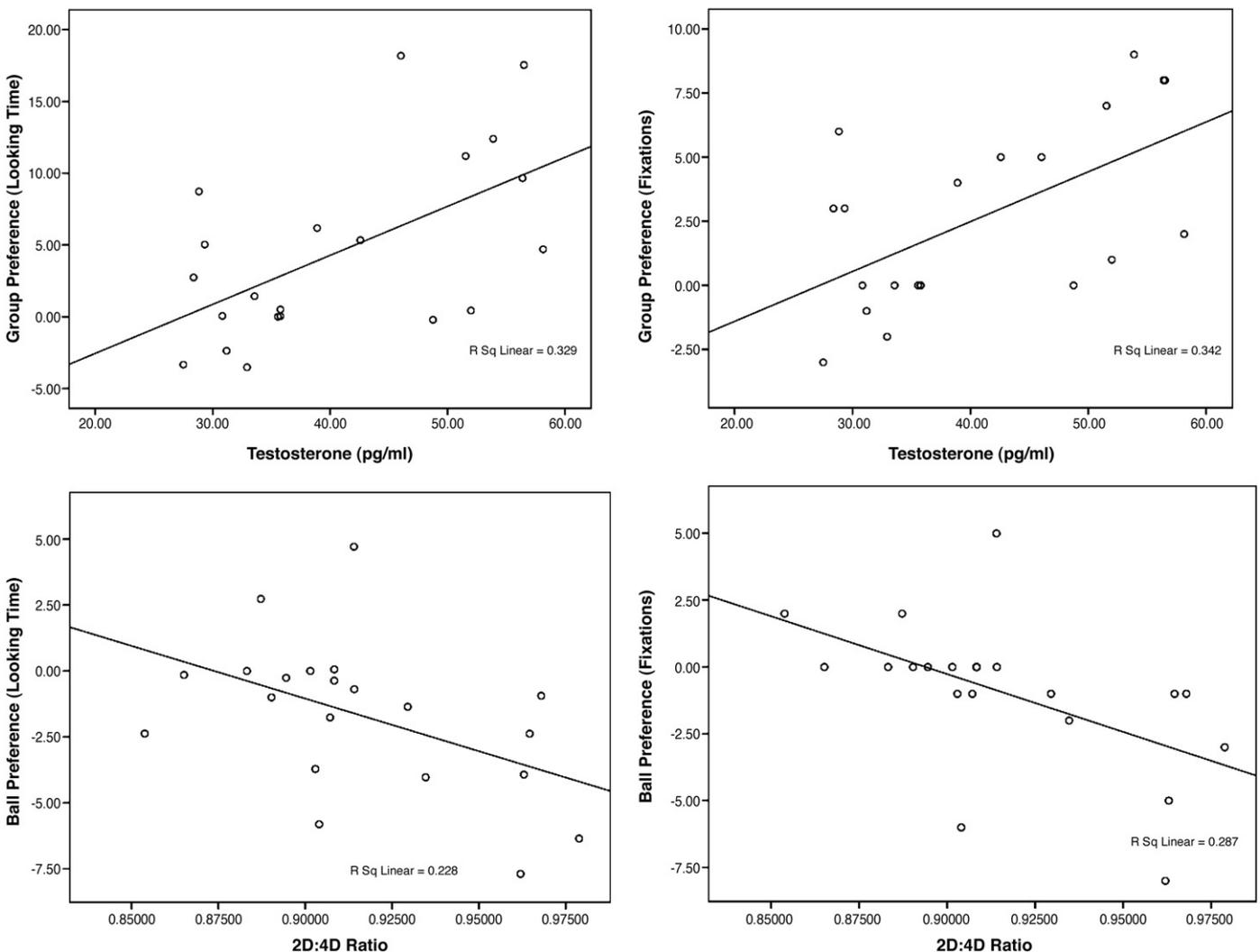

Fig. 2. Hormone–behavior associations and digit-ratio-behavior associations in male infants ($n = 21$).

are consistent with the hypothesis that activation of the HPG axis in postnatal life contributes to the development of sex differences in behavior, a possibility suggested by previous evidence of the biologic actions of hormones in prepubertal children (see Aksglaede et al., 2006) and recently observed sex differences in human behavior during the first year of life (Alexander et al., 2009; Moore and Johnson, 2008; Quinn and Liben, 2008). We have speculated previously that higher levels of androgens during a period of rapid brain development, including accelerated synapse formation in the visual cortex (Johnson, 2003), may organize visual processing pathways and result in a gender-linked bias for perceptual features (e.g., color, form, movement) that contributes to sex differences in play styles and spatial processing (Alexander, 2003). A similar argument has been put forth to explain sex differences in infants' visual preferences for groups of individuals (Benenson et al., 2007). The findings from the present investigation are consistent with this possibility, although further research is needed to address the specific perceptual features that underlie sex differences in visual interest and the precise neural mechanisms whereby androgens may influence the development of such preferences.

In contrast to the results of previous research on older infants (Alexander et al., 2009; Benenson et al., 2004), no sex differences in preferences for toys or groups were observed in these 3- to 4-month-old infants. The relatively small number of infants in this research may explain this finding. However, sex-linked visual preferences for toys in older infants were documented using a smaller sample size (17 boys and 13 girls) (Alexander et al., 2009). It may be that, if hormones in early infancy influence the expression of a visual preference for such stimuli, then the point of divergence in male and female visual response to such stimuli might be expected to follow the period of heightened HPG activation. Consistent with this possibility and for a role of postnatal testosterone in the formation of such preferences, higher testosterone levels in male infants predicted stronger preferences for the group of figures. Although a similar association between salivary testosterone and preferences for the male-typical toy was not observed, our finding that smaller digit ratios indicating higher prenatal androgen action (McIntyre, 2006) predicted stronger preferences for the ball compared to the doll is consistent with other evidence of the masculinizing and defeminizing effects of prenatal androgens on children's play preferences (Hines and Kaufman, 1994). Therefore, one possible explanation for our pattern of hormone–behavior associations in male infants is that prenatal androgen levels may be important for the early organization of preferences for object features that characterize male-preferred and female-preferred toys, whereas postnatal androgen levels may be important for the early organization of preferences for larger social groups. Interestingly, the hypothesis that postnatal androgens contribute to the organization of male-typical behavior (Mann and Fraser, 1996) has not been supported in research on non-human primates (e.g., Wallen et al., 1995; Brown and Dixson, 1999). However, our findings and the results of recent research showing levels of serum testosterone in early infancy are associated with brain activation patterns during auditory processing (Friederici et al., 2008) suggest that the role of the transient activation of the HPG axis on behavior varies across primate species.

Our findings have clear theoretical and applied implications. An earlier association between estradiol levels and breast size in infant girls suggested a "developmental window" when tissue stimulation becomes significant for later breast development in adulthood (Schmidt et al., 2002). In an analogous manner, we suggest that our novel findings indicate a developmental window for the organization of brain systems that may become significant for the development of later gender-linked behavior in males. For example, our results suggest that hormone levels may influence male behavioral response to socialization in early life. Finally, researchers have long appreciated that endocrine disorders or administration of hormones during prenatal life may alter the typical development and expression of male and female behavior. Our findings implicating the postnatal period in the development of sex differences in behavior suggest recent concerns that endocrine disruptors (e.g., phylates, pesticides, growth hormone) in infancy contribute to atypical sexual development (Aksglaede et al., 2006 for review) may extend beyond physiological events (such as precious puberty and hormone-related cancers) to the realm of human behavior.

Acknowledgments

References

Aksglaede, L., Juul, A., Leffers, H., Skakkeback, N.E., Andersson, A.-M., 2006. The sensitivity of the child to sex steroids: possible impact of exogenous estrogens. Hum. Reprod. Update. 12, 341–349.

Alexander, G.M., 2003. An evolutionary perspective of sex-typed toy preferences: pink, blue and the brain. Arch. Sex. Behav. 32, 7–14.

Alexander, G.M., Hines, M., 1994. Gender labels and play styles: their relative contribution to children's selection of playmates. Child Dev. 65, 869–879.

Alexander, G.M., Hines, M., 2002. Sex differences in responses to children's toys in a non-human primate (Cercopithecus aethiops sabaeus). Evol. Hum. Behav. 23, 467–479.

Alexander, G.M., Wilcox, T., Woods, R., 2009. Sex differences in infants' visual interest in toys. Arch. Sex. Behav. 48, 427–433.

Benenson, J.F., Duggan, V., Markovits, H., 2004. Sex differences in infants' attraction to group versus individual stimuli. Infant Behav. Dev. 27, 173–180.

Benenson, J.F., Markovits, H., Muller, I., Challen, A., Carder, H.P., 2007. Explaining sex differences in infants' preferences for groups. Infant Behav. & Dev. 30, 587–595.

Breedlove, S.M., Cooke, B.M., Jordan, C.L., 1999. The orthodox view of brain sexual differentiation. Brain Behav. Evol. 54, 8–14.

Brown, G.R., Dixson, A.F., 1999. Investigation of the role of postnatal testosterone in the expression of sex differences in behaviour in infant rhesus macaques (Macaca mulatta). Horm. Behav. 35, 186–195.

Chellakooty, M., Schmidt, I.M., Haavisto, A.M., Boisen, K.A., Damgaard, I.N., Mau, C., et al., 2005. Inhibin A, inhibin B, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels in 473 healthy infant girls. The J. Endocrinol. Metab. 88, 3515–3520.

Cohen, J., 1977. Statistical Analysis for the Behavioral Sciences. Academic Press, New York.

Collaer, M.L., Hines, M., 1995. Human behavioral sex differences: a role for gonadal hormones during early development? Psychol. Bull. 118, 55–107.

Davidson, J.M., Camargo, C.A., Smith, E.R., 1979. Effects of androgen on sexual behavior in hypogonadal men. J. Clin. Endocrinol. Metab. 48, 955–958.

de Ronde, W., van der Schouw, Y.T., Pierik, F.H., Pols, H.A., Muller, J., Grobbee, D.E., Gooren, L.J.G., Weber, R.F.A., de Jong, F.H., 2005. Serum levels of sex hormone-binding globulin (SHBG) are not associated with lower levels of non-SHBG-bound testosterone in male newborns and healthy adult men. Clin. Endocrinol. 62, 498–503.

Forest, M.G., Sizonenko, P.C., Cathiard, A.M., Bertrand, J., 1974. Hypophysogonadal function in humans during the first year of life: 1. Evidence for testicular activity in early infancy. J. Clin. Invest. 53, 819–828.

Friederici, A.D., Pannekamp, A., Partsch, C.-J., Ulmen, U., Oehler, K., Schmutzler, R., Hesse, V., 2008. Sex hormone testosterone affects language organization in the infant brain. NeuroReport 19, 283–286.

Hassett, J.M., Siebert, E.R., Wallen, K., 2008. Sex differences in rhesus monkey toy preferences parallel those of children. Horm. Behav. 52, 359–364.

Hines, M., 2004. Brain Gender. Oxford University Press, Oxford.

Hines, M., Kaufman, F.R., 1994. Androgen and the development of human sex-typical behavior: rough-and-tumble play and sex of preferred playmates in children with congenital adrenal hyperplasia (CAH). Child Dev. 65, 1042–1053.

Huhtaniemi, I., Dunkel, L., Perheentupa, J., 1986. Transient increase in postnatal testicular activity is not revealed by longitudinal measurements of salivary testosterone. Pediatr. Res. 20, 1324–1327.

Johnson, M.H., 2003. The development of human brain function. Biol. Psychiatry 54, 1312–1316.

Main, K.M., Schmidt, I.M., Skakkebaek, N.E., 2005. A possible role for reproductive hormones in newborn boys: progressive hypogonadism with the postnatal testosterone peak. J. Clin. Endocrinol. Metab. 85, 4905–4907.

Mann, D.R., Fraser, H.M., 1996. The neonatal period: a critical interval in primate development. J. Endocrinol. 149, 191–197.

McIntyre, M.H., 2006. The use of digit ratios as markers for perinatal androgen action. Reprod. Biol. Endocrinol. 4, 10–18.

Moore, D.S., Johnson, S.P., 2008. Mental rotation in human infants: a sex difference. Psychol. Sci. 11, 1063–1066.

Quinn, P.C., Liben, L.S., 2008. A sex difference in mental rotation in young infants. Psychol. Sci. 11, 1067–1070.

Schmidt, I.M., Chellakooty, M., Haavisto, A., Boisen, K.A., Damgaard, I.N., Steendahl, U., Toppari, J., Skakkebaek, N.E., Main, K.M., 2002. Gender difference in breast tissue size in infancy: correlation with serum estradiol. Pediatr. Res. 52, 682–686.

Sisk, C.L., Foster, D.L., 2004. The neural basis of puberty and adolescence. Nat. Neurosci. 7, 1040–1042.

Wallen, K., Maestripieri, D., Mann, D.R., 1995. Effects of neonatal testicular suppression with a GnRH antagonist on social behavior in group-living juvenile rhesus monkeys. Horm. Behav. 29, 322–337.