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Fetal testosterone and empathy: Evidence from the Empathy Quotient (EQ) and the ''Reading the Mind in the Eyes'' Test

Emma Chapman, Simon Baron-Cohen, and Bonnie Auyeung
University of Cambridge, Cambridge, UK

Rebecca Knickmeyer
University of Cambridge, Cambridge, UK, and University of North Carolina, Chapel Hill, NC, USA

Kevin Taylor and Gerald Hackett
Addenbrooke's Hospital, Cambridge, UK

Empathy involves an understanding of what others are thinking and feeling, and enables us to interact in the social world. According to the Empathizing–Systemizing (E–S) theory, females on average have a stronger drive to empathize than males. This sex difference may in part reflect developmental differences in brain structure and function, which are themselves under the influence of fetal testosterone (fT). Previous studies have found that fT is inversely correlated with social behaviors such as eye contact in infancy, peer relationships in preschoolers, and mentalistic interpretation of animate motion. Male fetuses are exposed to higher levels of testosterone than are female fetuses. The present study investigates empathizing in children, as a function of amniotic measures of fT. One hundred ninety-three mothers of children (100 males, 93 females) aged 6–8 years of age completed children's versions of the Empathy Quotient (EQ-C), and the children themselves were tested on ''Reading the Mind in the Eyes'' Task (Eyes-C). All mothers had had amniocentesis during the 2nd trimester of pregnancy. There was a significant negative correlation between fT and scores on both measures. While empathy may be influenced by post-natal experience, these results suggest that pre-natal biology also plays an important role, mediated by androgen effects in the brain. These results also have implications for the causes of disabilities involving empathy, such as autism spectrum conditions, and may explain the increased rate of such conditions among males.

Fetal testosterone (fT) and development

From the earliest stages of pre-natal life, gonadal hormones influence sexual differentiation of both the body and brain (Abramovich, 1974; Dorner, 1978). These include the estrogens (e.g., estradiol), progestins (e.g., progesterone) and androgens (e.g., testosterone). At conception, karyotype determines whether the fetus is male (XY) or female (XX). This chromosomal difference leads to differentiation of the gonads as

Correspondence should be addressed to: Emma Chapman, Autism Research Centre, University of Cambridge, Douglas House, 18b Trumpington Road, Cambridge CB2 2AH, UK. E-mail: elc36@cam.ac.uk

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ovaries or testes, due to the presence or absence of the SRY gene (Fechner, 1996). This differentiation results in different hormonal environments between male and female fetuses. Both sexes are exposed to androgens *and* estrogens, but overall production of these hormones and the number of receptors for them, differ markedly between the sexes (Kucinskas & Just, 2005). This disparity appears to drive much subsequent sexual differentiation (Fuchs & Klopper, 1983; MacLusky & Naftolin, 1981; Wilson, Foster, Kronenberg, & Larsen, 1998). In human male fetuses, the differentiated testes start producing androgens such as testosterone. By week 8 of gestation, testosterone levels have risen to a significantly higher level in male fetuses than female fetuses and they continue to rise and peak at around week 16 (Smail, Reyes, Winter, & Faiman, 1981). After this time testosterone levels decline until week 24 of gestation in male fetuses, and the large sex difference disappears (Fechner, 1996). This critical window of time is, however, long enough to ensure that physical sexual differentiation occurs.

Sexually dimorphic behavior and cognition

Beyond physical sexual differentiation, there are also sex differences in behavior, e.g., aggression and play preferences, and cognition, e.g., spatial ability, targeting skills, and social skills (Collaer & Hines, 1995; Kimura, 1996; Nicholson & Kimura, 1996). The Empathizing–Systemizing (E–S) theory of psychological sex differences proposes that on average, empathizing is stronger in females (Baron-Cohen, 2003). ''Empathizing'' is the drive to identify another person's emotions, thoughts and intentions. Empathy also involves having an appropriate emotional reaction in response to the other person's emotion. A study by Baron-Cohen, Wheelwright, Hill, Raste, and Plumb (2001a) used the ''Reading the Mind in the Eyes'' test (Eyes Test) to examine subtle mental state and complex emotion recognition in adults. In this task the participant is presented with a series of photographs of the eye region of the face, and is asked to choose which of four words best describes what the person in the photograph is thinking or feeling. Females scored significantly higher than males on this task.

A related measure is the ''Empathizing Quotient'' (EQ). This self-report questionnaire contains 40 empathy items and 20 filler/control items. On each empathy item, a person can score 2, 1, or 0, so the EQ has a maximum score of 80 and a minimum of zero. A person who scores highly on this test would be considered a good empathizer: easily able to detect, and be appropriately affected by, other people's feelings. A study by Baron-Cohen and Wheelwright (2004) looked at performance on the EQ in 197 adults of normal-range intelligence from a general population. Women scored significantly higher than men on the EQ. Taken together, these results suggest that, on average, women have a stronger drive to empathize. This has been further confirmed in other samples (Baron-Cohen, Richler, Bisarya, Gurunathan, & Wheelwright, 2003; Wheelwright et al., 2006) and using other empathizing tasks (Baron-Cohen & Wheelwright, 2003; Golan, Baron-Cohen, & Hill, 2006).

The precursors to such sex differences are evident from the very first few days of life. A study involving one-day-old newborns looked at preferences for looking at particular stimuli (Connellan, Baron-Cohen, Wheelwright, Ba'tki, & Ahluwalia, 2001). The stimuli were either social (a face) or mechanical (a mobile). It was found that female newborns spent more time looking at the face than the mechanical stimuli, while the opposite was true of male newborns. The fact that this difference is present from birth suggests the role of pre-natal biology.

fT and sexually dimorphic behavior and cognition

Psychological sex differences in the general population are likely in part to reflect differences in brain structure and function. In animal studies, fetal testosterone (fT) has been shown to affect the anatomy of specific brain areas, including the hypothalamus, limbic system, and neocortex (Geschwind & Galaburda, 1985). All of these regions express androgen receptors and are implicated in sexually dimorphic behaviors (Arnold & Gorski, 1984; Breedlove, 1984; MacLusky & Naftolin, 1981). Furthermore fT has been shown to directly affect related sexually dimorphic behaviors such as aggression, activity level and spatial navigation (Williams & Meck, 1991).

Several studies have looked at the effects of testosterone administration during pregnancy in animals. Pregnant rhesus monkeys injected with testosterone *early* in gestation give birth to daughters who had male genitalia externally, despite being *genetically* female. Later in infancy, these same daughters showed a more ‘‘male’’ style of play, showing more rough-and-tumble play, which could be considered to entail lower ‘‘empathy’’. When testosterone injections were administered *late* in gestation, daughters were genitally female but exhibited the same levels of masculine behaviors in infancy as the early testosterone group (Goy, Bercovitch, & McBrair, 1988).

Studies of this kind in humans are limited because it would be unethical to administer hormones during pregnancy as part of a research study. Other non-invasive approaches have therefore been employed. An opposite sex twin study showed that girls exposed to *natural* testosterone produced by their twin brothers had increased aggression post-natally, compared to same-sex female twins (Cohen-Bendahan, Buitelaar, van Goozen, Orlebeke, & Cohen-Kettenis, 2005). Aggression, and in particular direct aggression, is thought to require low levels of empathizing. Consistent with this, there is evidence for increased post-natal aggression in girls with congenital adrenal hyperplasia (CAH), compared to unaffected females (Berenbaum & Resnick, 1997). Females with CAH are exposed to elevated adrenal androgens during pre-natal development due to a specific enzyme deficiency. Females with CAH also show increased male-typical play preferences and decreased care-giving behavior, despite having been brought up as females since birth (Berenbaum, Duck, & Bryk, 2000).

Other human studies have taken advantage of *amniocentesis* in humans to assay fT, and then follow up the children to test if fT influences later behavior. One of the first such studies looked at mental rotation at age 7 years old in a Canadian sample and found this was positively correlated with fT (Grimshaw, Sitarenios, & Finegan, 1995). More recently, the Cambridge longitudinal study of the direct effect of fT on child development observed infants whose fT was measured in 2nd trimester amniotic fluid, collected during routine amniocentesis. At 12 months old, they found that girls exhibited higher levels of eye contact than boys overall, and that fT level was inversely correlated with the amount of eye contact (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2002a). When these infants were 24 months of age, it was found that girls had a larger vocabulary range than boys and that there was a negative correlation between fT and vocabulary level (Lutchmaya et al., 2002b).

At 48 months old, these infants were tested using the Children’s Communication Checklist (CCC; Bishop, 1998), which assesses quality of social relationships, and restricted interests. It was found that fT was again inversely correlated with quality of social relationships, but directly correlated with narrow interests (Knickmeyer, Baron-Cohen, Raggatt, & Taylor, 2005a). Finally, these same children were administered the test of mentalizing developed by Castelli, Happé, Frith, and Frith (2000), where the child is asked to describe the movement of geometric shapes presented on a computer screen. It was found that fT was inversely correlated with the frequency with which children used intentional language, including description of mental and emotional states (Knickmeyer, Baron-Cohen, Raggatt, Taylor, & Hackett, 2006).

The aim of the present study was to extend the Cambridge longitudinal study to investigate the relationship between levels of fT and empathizing in children at aged 6–9 years. Children completed two measures: child versions of the ‘‘Reading the Mind in the Eyes’’ Task (Eyes-C), and the Empathy Quotient (EQ-C). Consistent with previous studies, we predicted that fT level would be *inversely* correlated with performance on these tasks. We also predicted there would be a significant sex difference in average test scores, with girls scoring more highly on both the EQ-C and Eyes-C than boys.

EXPERIMENT 1: EMPATHIZING QUOTIENT—CHILD VERSION (EQ-C)

Methods

Participants

Participants for the EQ study were children (n = 193; 100 males, 93 females) aged 6.0 to 9.0 years. These children were part of a long-term study on the effects of fT. The mothers of all the children underwent amniocentesis in Cambridgeshire, Norfolk or Suffolk between June 1996 and June 1999. They all gave birth to healthy infants between December 1996 and December 1999. In any cases where multiple births resulted, these children were removed from the study owing to ambiguity over the identification of fT levels. The majority of mothers were referred for amniocentesis based on late maternal age (25%) or high results on the triple test (indicating an increased risk for Down's Syndrome; 60%). The remaining mothers underwent amniocentesis for several reasons including a family history of Down's or other chromosomal abnormalities seen on ultrasound scan. All amniotic samples were selected on the basis that they had tested negative for Down's and other chromosomal abnormalities. Evidence to date, shows that children for whom amniocentesis is carried out do not have a higher incidence of birth complications or impaired brain development relative to pregnancies without an amniocentesis (Finegan, Sitarenios, Bolan, & Sarabura, 1996).

Materials

The EQ-C is shown in Appendix 1. It comprises 27 questions requiring one of four responses: "definitely agree"; "slightly agree"; "slightly disagree"; "definitely disagree", with a 2-, 1- or 0-point scoring system. The maximal score any child could obtain on the EQ-C is therefore 54 points. The EQ-C was adapted from the adult EQ (Baron-Cohen & Wheelwright, 2004), by rephrasing questions to an age-appropriate level. The adult EQ has been shown to be a valid and reliable scale, with good test–retest reliability and positive correlations with other measures of empathy such as the Interpersonal Reactivity Index (IRI; Lawrence, Shaw, Baker, Baron-Cohen, & David, 2004). Normative data on the EQ-C was collected from 121 girls and 136 boys aged 6–12 years old (Auyeung, Baron-Cohen, Chapman, Knickmeyer, Taylor, & Hackett, submitted). These children were randomly selected from regional primary schools. The results of that study showed that in the age range 6–9 years old, boys scored a mean of 32.26 ($SD = 10.69$) and girls scored a mean of 36.88 ($SD = 11.41$). These results closely parallel those in the adult population, with females scoring significantly higher than males on this measure.

Procedure

A paper version of the EQ-C was sent by post to the parents of the children in the cohort. In the majority of cases, the mother was the primary caregiver. We requested that the mother fill out the EQ-C on behalf of their child. All questions had to be completed and each required a single definitive response to be counted. Any questionnaires with questions missing or multiple answers were excluded from our final analyses, unless we were able to contact the parent and complete the questionnaire.

Predictor variables

Fetal testosterone level (fT; nmol/l). The predictor of greatest interest in this project is fT. Testosterone levels in amniotic fluid were measured by radioimmunoassay by the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK, a method that our group has reported previously (Knickmeyer, Wheelwright, Taylor, Raggatt, Hacket, & Baron-Cohen, 2005b; Lutchmaya et al., 2002a, 2002b).

Sex of child. Boys were coded as 1 and girls were coded as -1 for all analyses.

Gestational age at amniocentesis (weeks). Levels of fT vary during gestation. Although amniocentesis on average takes place at week 16, it can occur anytime between week 12 and 22, according to individual circumstances. Thus gestational age at amniocentesis is an important variable to consider. Records were obtained from medical archives at the relevant hospital.

Sociodemographic variables

There are several social variables that could influence empathizing abilities in children. Relevant measures considered here are: maternal age, paternal age, number of siblings, maternal education and child's age

Results

There were significant differences between boys' and girls' fT levels, $t(190) = 10.4$, $p = .001$, $d = 1.85$. Equal variances were not assumed on any t-tests reported in this paper. The probability of a type I error was maintained at 0.05 for all t-tests.

We also looked at our lowest measures of fT in the sample to see if there was a floor effect at the detection limit (especially with the girls). There were no undetectable fT levels in either the boys or the girls and only 1 girl scored at the detection limit.

There was no significant difference between boys and girls for gestational age; $t(187) = -0.81$, $p = .42$, $d = 0.37$, maternal age; $t(189) = -0.08$,

$p = .94$, $d = 0.04$, or paternal age; $t(187) = -0.83$, $p = .41$, $d = 0.14$, or in number of siblings; $t(187) = -0.90$, $p = .37$, $d = 0.04$. Level of maternal education (rated on a 5-point scale: 1 = no formal qualifications, 2 = "O" Level/GCSE or equivalent, 3 = "A" Level, HND or vocational qualification, 4 = university degree, 5 = postgraduate qualification) showed a small but significant difference between boy and girls; $t(187) = 2.28$, $p = .03$, $d = 0.37$. There was no significant difference in age between males and females; $t(190) = 0.43$, $p = .39$, $d = 0.07$.

Table 1 shows means, standard deviations, and ranges for the outcome variables for each sex. We ran a residual analysis of fT and EQ-C scores to identify any outliers, of which there were three (1 boy and 2 girls), who were removed from the final data set. This left 190 children (99 boys and 91 girls) in our analyses. Kolmogorov–Smirnov tests indicated that none of the variable ranges were significantly skewed. We also looked at score distribution to investigate floor effects. None of the variables showed floor effects.

Relationship between outcome variables and fT levels

Table 2 shows the correlations between the outcome variable (EQ-C) and all predictor variables. The only predictor variables to significantly correlate with EQ-C score were fT and sex. We therefore used hierarchical regression analysis to examine the potential contribution of these predictor variables to the correlation with EQ-C score. In block 1, fT was entered and in block 2, sex was entered. The 3rd and final block looked at the interaction between fT and sex. Looking at fT in the 1st stage did not produce a significant *F* change: *F* change = 0.16, $\beta = .35$, $p = .41$. Inclusion of sex at the 2nd stage, however, did produce a significant *F* change: *F* change = 87, $\beta = -.63$, $p < .001$. This model explained 17% of the variance in EQ-C scores. The final stage revealed no significant *F* change according to a fT by sex interaction: *F* change = 4.86, $\beta = -.27$, $p = .11$. The only significant predictor in the final model was sex.

The effect of fT within each sex was then examined. In doing so the sample size for each analysis was halved and power thus reduced. Analyses revealed that there was a significant correlation between fT and EQ-C score for the boys; $r(99) = -.35$, $p = < .01$, but not for the girls (see Figure 1).

Discussion

A combined sex analysis showed there to be a significant negative correlation between fT level and performance on the EQ-C: $r(193) = -.28$, $p < .01$. However, there was also a significant difference between girls' and boys' EQ-C scores. Within-sex analyses revealed that there was a significant correlation between and fT and EQ-C score for the boys: $r(99) = -.35$, $p = < .01$, but not for the girls. The fact that a correlation is observed between fT and EQ-C for the boys may in part be due to a larger variation in fT levels for boys (0.10–2.05 nmol/l) compared to girls in this study (0.05–0.85 nmol/l). We investigated the influence of fT level and sex on EQ-C scores by running a stepwise analysis, which revealed a main effect of sex, but not fT in the final model. The strong correlation between sex and fT means

TABLE 1
Means, standard deviations, and ranges for EQ-C score predictor variables by sex

	Boys (n = 99)			*Girls (n = 91)*			
Variable	*Mean*	*SD*	*Range*	*Mean*	*SD*	*Range*	*Cohen's d*
EQ** (Max 58)	32.62	9.57	9–52	39.12	7.44	19–54	0.76
fT** (nmol/l)	0.81	0.37	0.10–2.05	0.31	0.18	0.05–0.85	1.85
Age of child	7.73	1.03	5.85–10.47	7.66	1.07	5.83–9.49	0.07
Gestational age at amnio (weeks)	16.21	1.34	13–20	16.78	1.61	13–22	0.37
Maternal age	41.02	4.78	29–52	41.19	4.59	29–51	0.04
Paternal age	42.10	5.89	31–56	42.90	5.60	34–62	0.14
Maternal education	3.47	1.21	1–5	3.07	0.95	1–5	0.37
Number of siblings	1.28	0.97	0–5	1.32	0.92	0–5	0.04

Note: *n* varies due to missing data for some participants. $*p < .05$; $**p < .01$.

TABLE 2

Correlation matrix showing relationships between the independent variables for all subjects of both sexes ($n = 190$) for the EQ-C

	EQ Score	*fT (nmol/l)*	*Child's age*	*Child's sex*	*Gestation age*	*Maternal age*	*Paternal age*	*Maternal education*	*No. of siblings*
EQ Score	—								
fT (nmol/l)	−0.28**	—							
Child's age	0.05	0.04	—						
Child's sex	0.30**	−0.65**	−0.04	—					
Gestation age	0.06	−0.02	0.13	0.08	—				
Maternal age	0.11	−0.17	0.09	0.02	−0.29**	—			
Paternal age	0.11	−0.16	0.23*	0.07	−0.15	0.59**	—		
Maternal education	−0.12	0.10	−0.01	−0.18*	−0.12	0.10	0.14	—	
No. of siblings	0.03	−0.02	0.09	0.02	−0.02	0.11	0.25**	−0.18*	—

Note: n varies due to missing data for some participants. Correlations are Pearson correlations. *$p < .05$; **$p < .01$.

that fT cannot be ruled out as a factor in producing the observed sex difference, but it is clear that the sex difference is larger than that which would be predicted by fT alone.

The significant difference seen between boys' and girls' EQ-C scores is consistent with the E–S theory of sex differences. This model predicts that, on average, females in the general population will score more highly than males on tests of empathy. The effect size of this sex difference was medium ($d = 0.76$), according to Cohen's guidelines. In Experiment 2, below, we aimed to test if the observed negative correlation between empathy and fT would be replicated using a very different test, the "Reading the Mind in the Eyes" task.

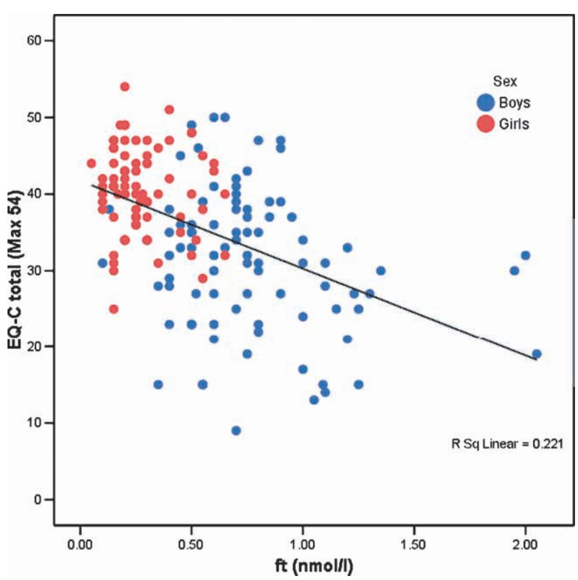

Figure 1. Graph showing relationship between EQ-C score and fT for both sexes.

EXPERIMENT 2: THE READING THE MIND IN THE EYES TASK—CHILD VERSION (EYES-C)

Methods

Participants

Participants for Experiment 2 were taken from the same cohort described in Experiment 1. We invited families from this group to come to Cambridge for cognitive testing and of those 78 children (40 boys, 38 girls) took part in the Eyes-C. All the children were 6–9 years of age.

Stimuli and materials

The Eyes-C task consisted of 28 pictures of the eye region of the face, each depicting a mental state, including subtle emotions. DMDX software (Forster & Forster, 2003) was used to run the task. Every picture was accompanied by 4 words, each describing an emotion, which was preceded by a number: 1, 2, 3 or 4. An example of a stimulus from the Eyes-C is shown in Appendix 2. The normative data was first reported in Baron-Cohen et al. (2001a), where it was found that in a sample aged 6–8 years old, males scored a mean of 7.3 ($SD = 0.7$), while females scored a mean of 6.8 ($SD = 0.6$). The task was run from an HP Pavilion ze4200 laptop connected to a 20-inch monitor, where the Eyes-C pictures were displayed, and a keyboard, where the participant's answers could be recorded.

Procedure

The child was seated comfortably and it was explained to them what they would be seeing on the computer monitor. The child sat 1 m from the screen and their chair was height adjusted to bring their eyes level with the center of the monitor. For each picture the child was told to first look at the picture of the eyes and then read all four words on the screen. They then had to choose the word that "best describes what the person in the picture is thinking or feeling." The children were told that if they had trouble understanding any of the words they should ask for help. Each child was also given the choice to either read the words for themselves, or for them to be read aloud by the experimenter. They answered by pressing the number at the top of the computer keyboard corresponding to the word they chose. There was no time limit to answer. After a choice was made, a central crosshair would appear on the screen for 500 ms before the next picture panel came up. Any child who had clear problems in completing the Eyes-C and who therefore scored at chance or below was excluded from the final data analyses.

In previous studies the children in our cohort were not yet old enough for standard IQ tests. In the present study we measured this in order to examine any relationships between IQ, fT and performance on empathizing tasks. This is particularly relevant to the Eyes-C task, as verbal IQ may facilitate understanding of more complex social words. The IQ test used was the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999).

Predictor variables

The predictor variables for Experiment 2 were the same as in Experiment 1.

Results

There was a significant difference between the boys' and girls' fT levels: $t(76) = 4.64$, $p = .001$, $d = 1.21$. There was not a significant difference in gestational age between the sexes: $t(76) = 0.93$, $p = .36$, $d = 0.07$. There was no significant difference between the sexes for maternal age, paternal age or number of siblings. However, there was a significant difference between the sexes for maternal education, with boys' mothers on average attaining a higher level than girls' mothers: $t(76) = 2.29$, $p = .03$, $d = 0.58$. There were no significant differences between boys' and girls' full IQ scores: $t(76) = -0.92$, $p = .19$, $d = 0.24$, or verbal IQ scores: $t(76) = 1.17$, $p = .15$, $d = 0.26$.

Table 3 shows means, standard deviations, and ranges for the outcome variables for each sex. A residual analysis of fT and Eyes-scores revealed 2 outliers (1 boy and 1 girl), which were removed from the data set. This left 76 children (39 boys, 37 girls) in our final analyses. Kolmogorov–Smirnov tests indicated that Eyes-C scores were significantly skewed. A logarithmic transformation reduced skewness to a non-significant level. None of the variables showed notable floor effects and according to calculations of binomial probability, both boys' and girls' average scores on the Eyes-C were above chance expectation (> 9).

TABLE 3
Means, standard deviations, and ranges for Eyes-C task predictor variables by sex

	Boys (n = 39)			Girls (n = 37)			
Variable	*Mean*	*SD*	*Range*	*Mean*	*SD*	*Range*	*Cohen's d*
Eyes-C (Max 28)	15.23	3.50	8–23	16.29	3.29	10–25	0.31
fT** (nmol/l)	0.79	0.41	0.13–1.95	0.38	0.27	0.05–1.00	1.21
Age of child	7.68	0.96	5.33–9.48	7.91	0.97	5.94–9.24	0.24
Gestational age at amnio (weeks)	16.14	1.60	13–20	16.05	1.03	14–18	0.07
Maternal age	41.49	5.46	29–52	40.64	4.48	30–48	0.17
Paternal age	41.99	6.37	31–56	43.42	6.29	35–59	0.23
Maternal education*	3.76	1.03	2–5	3.20	0.89	2–5	0.58
Number of siblings	1.61	1.05	0–5	1.33	0.88	0–5	0.29
IQ	99.23	10.08	80–118	101.79	11.48	80–119	0.24

Note: n varies due to missing data for some participants. *$p < .05$; **$p < .01$

Relationship between outcome variables and fT levels

Table 4 shows the correlations between the outcome variable (Eyes-C) and all predictor variables. The only predictor variables to significantly correlate with Eyes-C score at $p < .2$ were fT and child's age. We therefore forced these variables into a hierarchical regression analysis to investigate the potential contribution of these predictor variables to the correlation with Eyes-C score. Sex was also forced into the model as a suppressor variable at stage 1. Looking at child's age in the 1st stage did not produce a significant F change: F change $= 0.65$, $\beta = .03$, $p = .72$. Inclusion of fT at the 2nd stage produced a significant F change: F change $= 183$, $\beta = -.68$, $p = .001$. This model explained 39% of the variance in Eyes-C scores. The final stage revealed no significant F change according to an fT × child's age interaction: F change $= 2.76$, $\beta = -.27$, $p = .31$. The only significant predictor kept in the final model was fT. Within-sex analyses revealed that there was a significant negative correlation between fT and Eyes-C for *both* the boys: $r(38) = -.42, p = < .01$, and the girls: $r(34) = -.29$, $p = < .05$ (Figure 2).

Correlations in performance between Experiments 1 and 2

The final analysis looked at the relationship between individual EQ-C and Eyes-C scores. An overall group analysis revealed a significant positive correlation, $r(76) = .56$, $p = < .01$. This was similarly the case for both boys: $r(38) = .51$, $p = < .01$, and girls: $r(34) = .47$, $p = < .01$.

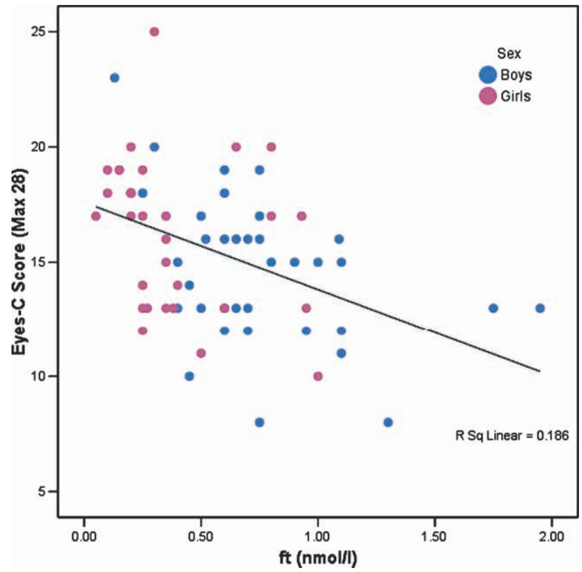

Figure 2. Graph showing relationship between Eyes-C score and fT for both sexes.

Discussion

A combined-sex analysis revealed a significant negative correlation between fT and Eyes-C score: $r(76) = -.43$, $p = < .01$. Within-sex analyses revealed that there was also a significant negative correlation between fT and Eyes-C for both the boys: $r(38) = -.42$, $p = < .01$, and the girls; $r(34) = -.29$, $p = < .05$. These combined- and within-sex correlations suggest a relationship between fT and empathizing. The stronger correlation observed between fT and Eyes-C for the boys may reflect the much larger variation in fT levels for boys compared to girls who took part: 0.13–1.95 and 0.05–1.00 nmol/l, respectively.

TABLE 4

Correlation matrix showing relationships between the independent variables for all subjects of both sexes ($n = 76$) for the Eyes-C task

Eyes-C score	*fT (nmol/l)*	*Child's age*	*Child's sex*	*Gestation age*	*Maternal age*	*Paternal age*	*Maternal education*	*No. of siblings*	*Child's IQ*
Eyes-C score	—								
FT (nmol/l)	−0.43**	—							
Child's age	0.29*	−0.25*	—						
Child's sex	0.16	−0.51**	0.12	—					
Gestation age	−0.18	0.03	0.22	−0.03	—				
Maternal age	0.11	−0.19	0.12	−0.08	−0.35**	—			
Paternal age	0.14	−0.22	0.22	0.11	−0.18	0.73**	—		
Maternal education	−0.05	0.09	−0.03	−0.28*	0.03	−0.01	−0.08	—	
No. of siblings	−0.05	0.01	0.12	−0.14	0.13	0.29*	0.23	−0.16	—
Child's IQ	0.15	−0.01	0.14	−0.12	−0.12	−0.06	−0.05	0.02	−0.04

Note: n varies due to missing data for some participants. Correlations are Pearson correlations. *$p < .05$; **$p < .01$.

Although girls tended to score higher than boys on the Eyes-C, there was no significant difference in scores ($d = 0.31$), as we had predicted.

The lack of a significant sex difference on the Eyes-C could be due to limited numbers of participants. Assuming the mean difference in our sample reflects that in the general population, a sample size of 82 would be required to give the model a power of 0.8. Our final sample size was almost as big as this ($n = 76$). Another factor to consider is the extent of overlap in fT levels within our cohort. The participants that took part in the Eyes-C were much closer in mean and range of fT levels ($d = 1.21$), than that observed in the general population ($d = 2.7$) (Finegan et al., 1989) and in Experiment 1 ($d = 1.85$), where a sex difference in task performance was seen.

There was also a correlation between Eyes-C score and child's age. The Eyes-C relies on understanding of emotional words and the difference in age of the children in this study was as much as three years in some cases. Over childhood, verbal ability continues to develop and there is evidence to show that neural processes underlying emotion recognition also change with age (Batty & Taylor, 2006; Herba & Phillips, 2004). The correlation between Eyes-C score and age may therefore be unsurprising. However, verbal IQ did not correlate with performance on this task, and children are capable of recognizing both basic and at least some complex emotions by 6 years of age (Saarai & Harris, 1990).

Hierarchical regression analysis revealed a main effect of fT in the final model, while child's age was excluded. Exclusion of an fT $\times$ child's age interaction indicates that the relationship between fT and Eyes-C score is the same across all ages. This suggests that performance on the Eyes-C is more strongly related to fT level than to age. Experiment 2 therefore provides evidence for the role of fT in the ability to recognize emotional expressions from the eye regions of the face. While there was no sex difference in performance in Experiment 2, this may have been due to the degree of overlap between boys and girls fT levels in this sample.

GENERAL DISCUSSION

The aim of this study was to establish whether fT is related to two measures of empathizing in typically developing children. Our measures included child versions of the Empathy Quotient (EQ-C) and the "Reading the Mind in the Eyes" Task (Eyes-C). We predicted that fT level would be inversely correlated with performance on both of these tasks. We also predicted that females would score significantly higher on both the EQ-C and the Eyes-C. In general, the evidence supported both predictions. It is of interest that the EQ and Eyes task are correlated in adult populations (Lawrence et al., 2004). Our study provides evidence that this is also the case in children. Our findings provide direct support for the argument that pre-natal biology influences post-natal social behavior.

Sex differences in behavior partly reflect sex differences in the brain. The EQ brings together the two major components of empathy (cognitive and affective empathy) and these depend on different, specific regions of the brain (Vollm, Taylor et al., 2006). The amygdala, for example, has been shown to be active during the processing of emotional states, and lesions to this area cause impairments of emotion recognition (Adolphs, 2002; Shaw, Bramham, Lawrence, Morris, Baron-Cohen, & David, 2005). This is also observed for the fusiform gyrus (FG; Adolphs, 2002). Emotion recognition from the eye region alone has also been shown to involve the amygdala (Adolphs, Baron-Cohen, & Tranel, 2002; Morris, deBonis, & Dolan, 2002) and the medial prefrontal cortex (MPFC; Baron-Cohen et al., 1999). All of these regions of the brain contain an abundance of androgen receptors through which fT acts (Goldstein et al., 2001). Furthermore, these regions of the brain have been shown to be functionally and structurally sexually dimorphic (Giedd, Castellanos, Rajapakse, Vaituzis, & Rapoport, 1997; Canli, Desmond, Zhao, & Gabrieli, 2002). A recent fMRI study using the Eyes test in typical males and females found that females showed more bilateral activity in the medial frontal cortex compared to males, while males showed more left-hemispheric activity in the superior temporal gyrus, compared to females (Baron-Cohen et al., 2006).

The results of the two experiments reported here have implications for our understanding of neurodevelopmental conditions such as autism. Autism and Asperger syndrome (AS) form part of the autism spectrum conditions (ASC) and are characterized by social and communication difficulties and repetitive and restricted behavior (American Psychiatric Association, 1994). They are more prevalent in males, with a ratio of 4 males to 1 female for autism, and as high as 9 males to 1 female for AS (Wing, 1981). Individuals with ASC perform poorly on tests of empathy, such the ''Reading the Mind in the Eyes Task'' or the EQ. People with ASC perform at a significantly lower level on this task than typical males, who in turn perform significantly lower than typical females. This has been found in both adult and child populations (Baron-Cohen, Richler et al., 2003; Baron-Cohen & Wheelwright, 2004; Baron-Cohen, Wheelwright, & Hill, 2001; Baron-Cohen, Wheelwright, Scahill, Lawson, & Spong, 2001b).

This extreme of the typical male pattern also extends to brain development and function in autism. One striking example is the amygdala, which as discussed before has been strongly implicated in emotion recognition and other tasks relevant to empathizing. During childhood, the amygdala is significantly larger in typically developing males than in females (Caviness, Kennedy, Richelme, Rademacher, & Filipek, 1996). There is evidence that the amygdala is abnormally large in children with autism even when corrected for total brain volume (Mosconi, 2005). Furthermore, this enlargement persists throughout the period of sex-differential amygdala growth observed in normal boys (Schumann, Hamstra et al., 2004; Sparks, Friedman et al., 2002). There is also evidence that relative to normal males and females, individuals with ASC show hypoactivity of the amygdala and the FG during tasks involving emotion recognition (Schultz, Grelotti et al., 2003; Wang, Dapretto, Hariri, Sigman, & Bookheimer, 2004).

If normal sex differences in empathy are mediated by fT, and autism is an exaggeration of the male pattern (Baron-Cohen, 2002), autism may also be related to fT. Indeed, there is evidence that ASC may involve elevated fT: (a) The ratio of the lengths of the 2nd and 4th digit (2D:4D) is negatively correlated with the ratio of fT to fetal estrogen (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004), and individuals with autism have lower 2D:4D ratios than people without autism spectrum conditions (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001). (b) Individuals with CAH, who have over-production of fT, show more autistic traits as measured by the Autism Quotient (Knickmeyer et al., 2006). (c) Levels of fT are positively correlated with repetitive and restricted interests in children, which are diagnostic criteria for ASC (Knickmeyer et al., 2005a). (d) Levels of fT are negatively correlated with eye contact and vocabulary development (Lutchmaya et al., 2002a, 2002b). Children who later go on to be diagnosed with classic autism score lower on these measures in the second year of life (Swettenham, Baron-Cohen, Charman, Cox, Baird, & Rees, 1998). (e) Individuals with autism may show precocious puberty, which may be influenced by fT (Mouridsen, 1989; Tordjman & Ferrari, 1992). (f) Finally, the number of autistic traits typically developing individuals exhibit is directly correlated with fT (Auyeung, Baron-Cohen, & Wheelwright, in press).

Limitations of the current studies

As with all studies involving amniocentesis, there are problems in determining the exact influence and mechanism of action of hormones upon the developing fetus. We have assumed that the fT levels measured are representative of the serum levels to which the fetal brain is exposed; an assumption that it has not been feasible to test directly as yet. Another limitation of the current study involves the demographic characteristics of our cohort. Mothers who undergo amniocentesis do so because of the possible risk of fetal abnormalities due to late maternal age or other risk factors. In any cases where fetal abnormalities were detected, these children were excluded. There is currently no evidence that amniocentesis or factors associated with it such as late maternal age or high AFP levels have any bearing on fT levels. Even if such a relationship existed, this is shared by the entire cohort, and this study measured the relative effects of fT. We did not find any relationship between fT and maternal age in this group. The possibility of parental bias in Experiment 1 is another limiting factor. While the EQ-C was designed to be as general as possible in terms of question content, there may be items that tend to be answered in a particular way for girls and boys. Evidence suggests that differences in cultural factors and socialization also have a role to play in the development of sex-typed behavior (Baenniger & Newcombe, 1989; Hyde, Fennema, Ryan, Frost, & Hopp, 1990; Newcombe & Dubas, 1992). Our study suggests that pre-natal biology has a role to play in social development, but this does not rule out the possibility that such effects are modified by post-natal experience.

CONCLUSIONS

The finding of similar significant *negative* correlations between fT and two independent tests of empathizing (the EQ-C, and the Eyes-C) suggests that there is a real effect of fT on performance on these psychological tests. The fact that fT was not correlated with IQ shows that this is a highly specific effect. The finding that non-social skills such as mental rotation (Grimshaw et al., 1995) and narrow interests (Knickmeyer et al., 2005a) show *positive* correlations with fT are in the direction that one would predict. We recognize that empathy is likely to be influenced by post-natal experience, independent of pre-natal biology, since it is known that early neglect and abuse reduce later empathy (Bowlby, 1978; Fonagy, 1998; Gordon, 2003). But the current studies provide converging evidence from two very different tests of empathy for the role of fT in shaping sex differences in this aspect of social cognition.

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APPENDIX 2

An example of stimuli from the Eyes-C task (Correct answer: interested.)

APPENDIX 1

The EQ-C Cambridge Child Personality Questionnaire Please complete by ticking the appropriate box for each statement

		Definitely agree	*Slightly agree*	*Slightly disagree*	*Definitely disagree*
1.	My child likes to look after other people.	☐	☐	☐	☐
2.	My child often doesn't understand why some things upset other people so much.	☐	☐	☐	☐
3.	My child would not cry or get upset if a character in a film died.	☐	☐	☐	☐
4.	My child is quick to notice when people are joking.	☐	☐	☐	☐
5.	My child enjoys cutting up worms, or pulling the legs off insects.	☐	☐	☐	☐
6.	My child has stolen something they wanted from their sibling or friend.	☐	☐	☐	☐
7.	My child has trouble forming friendships.	☐	☐	☐	☐
8.	When playing with other children, my child spontaneously takes turns and shares toys.	☐	☐	☐	☐
9.	My child can be blunt giving their opinions, even when these may upset someone.	☐	☐	☐	☐
10.	My child would enjoy looking after a pet.	☐	☐	☐	☐
11.	My child is often rude or impolite without realizing it.	☐	☐	☐	☐
12.	My child has been in trouble for physical bullying.	☐	☐	☐	☐
13.	At school, when my child understands something they can easily explain it clearly to others.	☐	☐	☐	☐
14.	My child has one or two close friends, as well as several other friends.	☐	☐	☐	☐
15.	My child listens to others' opinions, even when different from their own.	☐	☐	☐	☐
16.	My child shows concern when others are upset.	☐	☐	☐	☐
17.	My child can seem so preoccupied with their own thoughts that they don't notice others getting bored.	☐	☐	☐	☐
18.	My child blames other children for things that they themselves have done.	☐	☐	☐	☐
19.	My child gets very upset if they see an animal in pain.	☐	☐	☐	☐
20.	My child sometimes pushes or pinches someone if they are annoying him/her.	☐	☐	☐	☐
21.	My child can easily tell when another person wants to enter into conversation with him/her.	☐	☐	☐	☐
22.	My child is good at negotiating for what they want.	☐	☐	☐	☐
23.	My child would worry about how another child would feel if they weren't invited to a party.	☐	☐	☐	☐
24.	My child gets upset at seeing others crying or in pain.	☐	☐	☐	☐
25.	My child likes to help new children integrate in class.	☐	☐	☐	☐
26.	My child has been in trouble for name-calling or teasing.	☐	☐	☐	☐
27.	My child tends to resort to physical aggression to get what they want.	☐	☐	☐	☐