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Short communication

Foetal testosterone and eye contact in 12-month-old human infants

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Abstract

Amniotic fluid was analysed for foetal testosterone (FT) level. Postnatally, the infants (29 girls and 41 boys) and parents were filmed at 12 months of age, and the amount of eye contact made by the infant to the parent was recorded. Girls made significantly more eye contact than boys. This replicates previous studies showing a female superiority in sociality more broadly, and eye contact in particular. The amount of eye contact varied quadratically with foetal testosterone level when data from both sexes was examined together, and when the data for the boys was examined alone. This suggests that foetal testosterone may shape the neural mechanisms underlying social development.

Keywords: Foetal testosterone; Eye contact; Infants

Foetal testosterone (FT) acts on the developing brain to influence cerebral lateralisation (Grimshaw, Bryden, & Finegan, 1995b). The evidence for this comes from at least four sources: (1) animal studies demonstrate that prenatal exposure to male sex hormones organises the brain for post-natal masculine behaviour in a number of species (Arnold & Gorski, 1984; Harris & Levine, 1962; Williams, Barnett, & Meck, 1990); (2) human studies demonstrate that abnormal hormonal environments during pregnancy affect foetal and later development. One example is congenital adrenal hyperplasia (CAH), a genetic defect of the adrenal cortex which causes over-production of foetal androgens. Affected females show masculinisation compared to their unaffected siblings (Collaer & Hines, 1995); (3) Geschwind's 1985 theory

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(Geschwind & Galaburda, 1985a, 1985b, 1985c, 1987) proposed that elevated FT levels causes specific language impairment and autism. These are both primarily male conditions. His theory suggested FT affected both cerebral lateralisation and functional dominance (e.g., for language) in the brain; (4) some sexually dimorphic behaviour is influenced by gonadal hormones (Hines & Shipley, 1984). For example, mental rotation performance is better in males than in females and correlates significantly with FT (Grimshaw, Sitarenios, & Finegan, 1995a).

Sex differences have been found in social development (Baron-Cohen & Hammer, 1997; Baron-Cohen, Jolliffe, Mortimore, & Robertson, 1997; Baron-Cohen, O'Riordan, Jones, Stone, & Plaisted, 1999; Connellan, Baron-Cohen, Wheelwright, Ba'tki, & Ahluwalia, 2001). To date, no one has tested if this is related to foetal FT. The aims of the current study were to test if (a) there was a sex difference in the amount of eye contact at 12 months of age, as a marker of social development; and (b) amount of eye contact made by the child was related to FT levels.

Eye contact is of major importance in normal social development (Baron-Cohen, 1995; Stern, 1977; Trevarthen, 1979). Infants as young as 2 months of age spend more time looking at the eye region of the face than any other part of the face (Maurer & Salapatek, 1976). This may also be relevant to autism, which is not only more common in males, but is defined by marked social impairment, such as abnormal eye contact (Phillips, Baron-Cohen, & Rutter, 1992; Sigman, Mundy, Ungerer, & Sherman, 1986; Swettenham et al., 1998). One relevant theory claims that autism is an extreme form of the male brain (Baron-Cohen, 2000; Baron-Cohen & Hammer, 1997).

Amniocentesis, a routine antenatal test offered during pregnancy to mothers at high risk of carrying a foetus with Down Syndrome, is a way to access information about foetal hormones (Finegan, Bartleman, & Wong, 1991). The amniotic fluid from this test can be analysed for foetal hormone levels. The timing of the test is usually after the surge in testosterone production by the male foetus, around 10–12 weeks of gestation (Finegan et al., 1991). The surge coincides with the critical period for sexual differentiation of the brain (Abramovich & Rowe, 1973). The surge is absent in females, although females foetuses do produce testosterone from the adrenal glands (Martin, 1985).

The current study had three predictions: (1) girls would make more eye contact than boys (Podrouzek & Furrow, 1988); (2) amount of eye contact would be inversely related to FT levels (based on Geschwind); (3) a non-linear relationship between FT level and eye contact would result if, e.g., there was a different relationship within each sex (based on Grimshaw et al., 1995a).

Seventy mothers were recruited, who had undergone amniocentesis in the Cambridge region between June 1996 and June 1997 and who had given birth to healthy singleton infants (29 girls and 41 boys) between December 1996 and December 1997.[1] When their infant reached 12 months of age, parents were invited to bring them in to the lab to take part in a short play session. The infant's amniotic fluid sample was retrieved from frozen storage at Addenbrooke's Hospital, Cambridge, where FT levels were measured by radioimmunoassay, by a technician blind to the videotaped assessments.[2] For boys, the FT level ranged from 0.125 to 1.800 nmol/l with $M = 0.940$ nmol/l (SD $= 0.37$ nmol/l). For girls, the FT level ranged from 0.150 to 0.800 nmol/l with $M = 0.360$ nmol/l (SD $= 0.16$ nmol/l). For both sexes together, the FT level ranged from 0.125 to 1.8 nmol/l with $M = 0.701$ nmol/l (SD $= 0.41$ nmol/l).

The infants were filmed for approximately 20 min with one parent (the mother in 66 cases, and the father in 4 cases) and one female experimenter. During this time, infants were sequentially presented with six toys. A measure was obtained from the videotapes, by two independent judges blind to the FT data, of the number of times the infant made eye contact with the parent. This was expressed as the number of episodes over 20 min. Eye contact was defined as any occasion when the infant looked at the face region of the parent. We did not attempt to assess when true eye contact had occurred, since looking up at the face can be coded far more reliably and has been found to correlate with this (Kleinke, 1986). We examined rate of eye contact with the parent, not the experimenter, since the latter could have been confounded by shyness and reaction to strangers.

We also included the following eight control variables in the analysis. (1) Oestradiol in the amniotic fluid. This has feminising effects on development, so could be considered as an opposing influence to testosterone, and is synthesised in vivo via aromatisation of testosterone and related precursors (MacLusky & Naftolin, 1981). (2) Alpha-foetoprotein (αFP) in the amniotic fluid. This is thought to be a general marker for severe foetal ill-health and also provides a specific control for any unexpected abnormalities of amniotic fluid dilution. (3) Sex of infant. (4) Number of siblings. (5) Maternal age. (6) Paternal age. (7) Foetal (gestational) age. (8) Educational level attained by the parents (on a 5-point scale: 1: no formal qualifications, 2: 'O' level/G.C.S.E. or equivalent, 3: 'A' level, HND or vocational qualification, 4: university degree, 5: postgraduate qualification. The score for both parents was added together). This descriptive data (for both sexes combined) is presented in Table 1.

Both frequency and duration of eye contact episodes were coded from the videotaped sessions. Frequency and duration were found to be significantly correlated to each other (Spearman's $\rho = .8$, $p < .01$). Frequency was considered to be a more accurate measure. Consequently, only frequency of eye contact is discussed here.

For boys, the number of eye contact episodes over 20 min ranged from 3.0 to 46.2, $M = 16.1$ (SD = 10.0). For girls the number of eye contact episodes over 20 min ranged from 3.8 to 55.0, $M = 22.0$ (SD = 12.1). For both sexes together the number of eye contact episodes over 20 min ranged from 3.0 to 55.0, $M = 18.6$ (SD = 11.3). The correlation between the scores recorded by the two judges was Pearson's $r = .91$. The standard error of estimate for the measure was $SE_{est} = 4.7$. As predicted, a significant sex difference (female superiority) was found for eye contact ($t = -2.2$, $p = .03$). See Figs. 1 and 2 for graphic summaries.

Table 1
Descriptive data for both sexes

	Both sexes ($n = 71$)			
	n[a]	Range	M	SD
FT (nmol/l)	70	0.13–1.8	0.7	0.4
Oestradiol level (pmol/l)	71	6.2–2630.0	922.1	400.0
AFP level (μmol/l)	71	3.1–23.6	10.6	4.0
Gestational age at amniocentesis (weeks)	59	14.0–21.0	16.6	1.7
Number of siblings	68	0.0–3.0	1.0	1.0
Maternal age at child's birth	71	24.0–46.0	35.5	4.6
Paternal age at child's birth	62	28.0–53.0	37.4	5.6
Level of education attained by parents	60	4.0–10.0	6.7	1.6

[a] This refers to the number of subjects for whom the data was available.

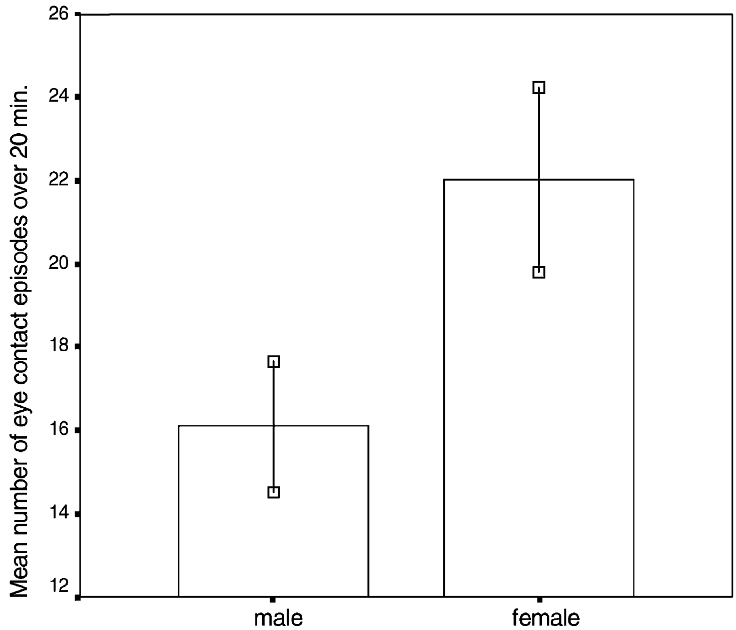

Fig. 1. Graph to show mean frequency of eye contact for each sex. Error bars represent the standard error of the mean.

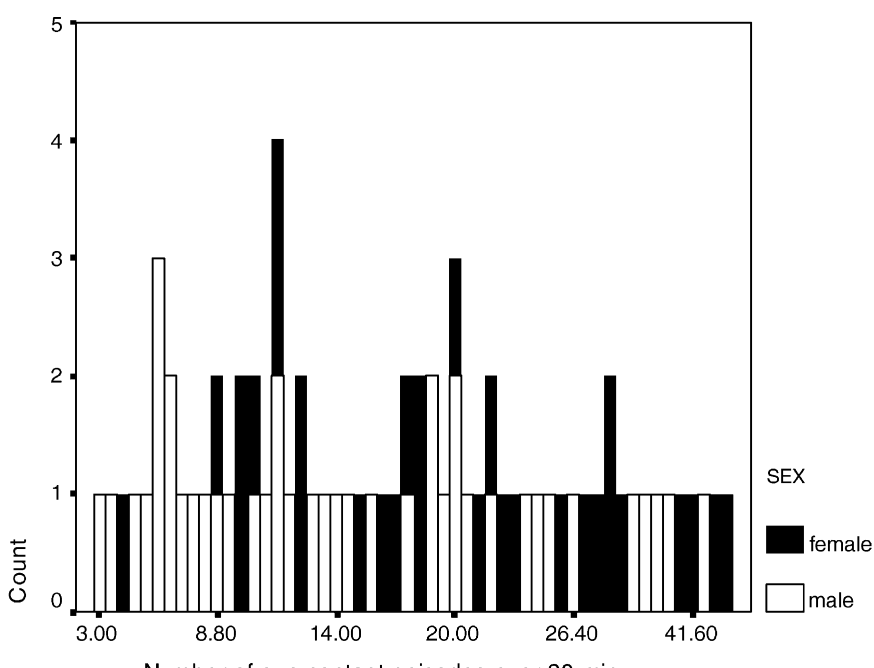

Fig. 2. Graph to show the distribution of eye contact scores for each sex.

We tested for correlations between eye contact and each of the predictor variables. If the relationship was significant at the $p < .2$ level, that predictor was entered into the model, together with any other predictor already found to be significantly correlated to it was also entered (Altman, 1991). The following predictors were found to be related to eye contact: FT level (Spearman's $\rho = -.3$, $p = .02$), sex (Mann–Whitney $U = 423.0$, $p = .03$), number of siblings (Spearman's $\rho = -.3$, $p = .03$), and parental education (Spearman's $\rho = -.2$, $p = .1$). The following predictors were also included in the model: paternal age, which was correlated to parental education, and maternal age, which was correlated to number of siblings. AFP and oestradiol were excluded from the model, as they were not significantly correlated to eye contact or to any of the predictors that were related to it. Because a quadratic relationship has been found in other studies (Grimshaw et al., 1995a), we investigated this statistically by including FT squared in the model.

Analyses were undertaken to investigate the inter-dependence, if any, between the predictor variables. The following relationships were found between them at the $p < .05$ level. FT level was significantly correlated to sex (Mann–Whitney $U = 78.5$, $p < .01$). Maternal age was significantly correlated to paternal age (Spearman's $\rho = .8$, $p < .01$) and to number of siblings (Spearman's $\rho = .3$, $p < .05$). Paternal age was significantly correlated to educational level attained by parents (Spearman's $\rho = .3$, $p < .05$).

A backward stepwise linear regression was used (entry criteria $p = .05$, removal criteria $p = .1$) to find the best fit for the dependence of eye contact on the predictor variables. The regression discarded from the model all predictors except FT and FT squared. In the low FT range, amount of eye contact decreased with FT. The opposite was true in the high FT range. In addition, the linear term for FT level was found to be a significant predictor of eye contact (see Table 2).

A significant quadratic relationship was found between eye contact and FT level when data from both sexes was examined together, when data from the boys was examined alone, but not for girls on their own (see Fig. 3).

We then undertook analysis of data within each sex (using the same procedure as described above), to find out if the previous result was simply due to a sex difference. The quadratic relationship was observed once again within boys, as was an inverse relationship with the linear term for FT (see Table 3). In addition, parental education level was retained in the model, but its effect did not reach significance. No significant relationship between FT and eye contact was observed within girls.

This study demonstrates a sex difference in the amount of eye contact between a parent and her 12-month-old infant (female infants showing more than male infants). This extends

Table 2
Regression model for both sexes together

Model	B	SE	Significance
Constant	.6	.2	.01
FT	−2.1	.7	.00
FT squared	1.0	.4	.01

Dependent variable: ln(eye contact) $R^2 = .2$.

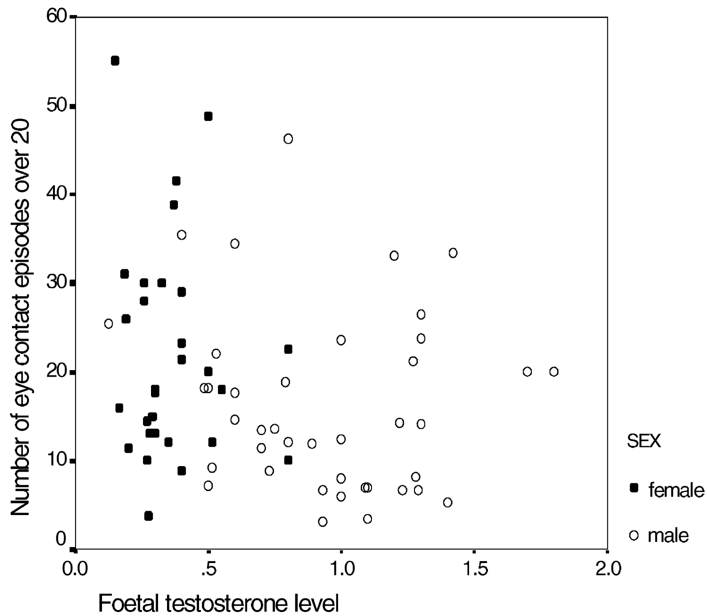

Fig. 3. Graph to show the relationship between FT level and eye contact for both sexes together.

previous results showing a female superiority in sociality more broadly (Maccoby, 1999), and in eye-contact specifically (Podrouzek & Furrow, 1988). This also extends our earlier finding that this sex difference is present very early in development, e.g., female neonates (24-h-old) look for longer at a face than at a mobile (Connellan et al., 2001). The present study also demonstrates that the amount of infant eye contact varies quadratically with amount of FT, as measured in amniotic fluid. This quadratic relationship was observed for both sexes together and, critically for the hypothesis, for boys alone. This result holds true even when factors such as sex, parental age and education and number of siblings are taken into account. Finally, the result holds true when data from boys and girls are analysed together, or when data from boys are analysed alone.

When the data from both sexes was examined together, the quadratic term for FT was a significant predictor of eye contact. Eye contact decreased with FT in the lower FT range,

Table 3
Regression model for boys only

Model	*B*	SE	Significance
Constant	1	.6	.01
FT	−2.5	1.01	.02
FT squared	1.2	.5	.02
Parental education level	−.1	.06	.06

Dependent variable: ln(eye contact) $R^2 = .3$.

and the opposite was true in the higher FT range. The linear term for FT was also a significant predictor of eye contact: eye contact decreased with increasing levels of FT. In order to investigate whether the quadratic relationship observed for both sexes together was simply describing a sex difference, it was necessary to remove sex as a variable and analyse the data within sex. The quadratic relationship was observed once again within boys. There was also an inverse relationship within boys between eye contact and the linear term for FT level.

No significant relationships between FT and eye contact were observed for girls. This may be because there were only 30 girls in the sample, making the resulting model under-powered. A sample size of approximately 60 would be required to give the model a power of 0.8, assuming a similar effect size as was detected when both sexes were examined together. It would be necessary to run this experiment with a larger sample of girls before drawing any strong conclusions.

This experiment has identified a link between prenatal hormone levels and social development at 12 months of age. The findings reported here have implications for future understanding of abnormal conditions of social development (such as autism) and for understanding the prenatal neurobiological organisation of social behaviour. Finally, the sex difference observed demonstrates that males and females differ in key social behaviours even at this very early age.

Notes

1. Reasons for the amniocenteses among the women in our sample were as follows: Triple test results (60%), late maternal age (25%); family history of Down Syndrome (3%); maternal anxiety (3%); ultrasound scan results (9%).
2. Assays were carried out by the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge. Amniotic fluid was extracted with diethyl ether. Recovery experiments have demonstrated 95% recovery of testosterone via this method. The ether was evaporated to dryness at room temperature and the extracted material redissolved in assay buffer. The testosterone was assayed by the DPC 'Count-a-Coat' method (Diagnostic Products Corp., Los Angeles, CA 90045-5597). This uses an antibody to testosterone coated onto propylene tubes and a 125-I labelled testosterone analogue. The detection limit of the assay is approximately 0.1 nmol/l. This method, thus, measures total extractable testosterone.

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