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In utero cortisol and testosterone exposure and fear reactivity in infancy

Kristin Bergman [a], Vivette Glover [a,*], Pampa Sarkar [a,b], Dave H. Abbott [c], Thomas G. O'Connor [d,*]

[a] Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK
[b] Department of Obstetrics and Gynaecology, Wexham Park Hospital, Slough, SL2 4HL,UK
[c] University of Wisconsin, Department of Obstetrics and Gynecology and Wisconsin National Primate Research Center, Madison, WI, USA
[d] Department of Psychiatry, University of Rochester Medical Center, 300 Crittenden Blvd, Rochester, NY, 14642, USA

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ABSTRACT

Fetal programming is emerging as a major conceptual model for understanding developmental origins of health and disease, including behavioral outcomes. As part of a larger study of prenatal stress and child development, we examined the association between prenatal hormone exposure and fear reactivity, a temperament dimension that is a predictor of long-term behavioral adjustment. Amniotic fluid was collected from a sample of women undergoing clinically indicated amniocentesis for later analysis of cortisol and testosterone. Children with normal birth outcomes were recalled for follow-up assessment at 17 months, at which time we administered an observational assessment of temperament (lab-TAB; $n = 108$). Information on pregnancy and obstetric outcome was included as covariates. Results indicated that there was a significant association between prenatal testosterone and observed fear reactivity in boys ($r(53) = 0.34$, $p = 0.01$); no significant effect was found in girls ($r(54) = -0.07$, ns); the effect remained when obstetric, psychosocial, and parental anxiety were controlled for. There was not a significant association between fetal cortisol exposure and fear reactivity. The prediction from in utero testosterone exposure to fear reactivity in boys extends prior research on prenatal testosterone and may represent an association with a general predisposition to greater arousal and reactivity.

There is considerable evidence, dating back many years, that prenatal steroid hormone exposure can have a lasting impact on health and development. One line of research, based on the organizational hypothesis (Phoenix et al., 1959), focuses particularly on the link between prenatal exposure to testosterone and sexual development and reproductive behavior. A somewhat separate line of research, based on a developmental programming model (Gluckman et al., 2008), examines how prenatal glucocorticoid exposure predicts offspring stress physiology and other outcomes. To a considerable extent, these findings rely on experimental animal work and assess a limited set of behaviors. In the current study, we extend existing research to human behavioral development, focusing on a key early marker of behavioral adjustment, fear reactivity, in a normative sample of infants.

A dominant hypothesis in research into the effects of prenatal androgen exposure proposed that prenatal androgens "organized" neural mechanisms serving reproductive and mating behavior (Phoenix et al., 1959). That is, the prenatal period constituted a particularly sensitive time in which exposure had organizing and lasting influence on neural mechanisms (even if it is not yet clear what those neural mechanisms are). Substantial evidence now indicates that prenatal testosterone shapes sexual development and reproductive behavior in animal models (Thornton et al., 2009); the applications to human development are not yet certain. There are, for example, several sets of findings linking prenatal testosterone to sex-typical play in children, with some but not others finding the effect limited to girls (Auyeung et al., 2009a; Ehrhardt and Meyer-Bahlburg, 1981; Hines et al., 2002). Another set of studies, derived in part from the hypothesis that prenatal testosterone may be associated with autistic-like traits (Auyeung et al., 2009b), shows that prenatal testosterone is associated with impaired social behavior (Knickmeyer et al., 2005) and poorer cognitive and language abilities (Jacklin et al., 1988; Knickmeyer et al., 2006). The extent to which prenatal testosterone may have effects that extend beyond these behavioral outcomes is not known.

A largely separate line of research that also proposes long-term effects of prenatal exposure on health and disease susceptibility is the developmental or fetal programming model. According to this model, the organism adapts to prenatal input and that this "set point" (e.g., in relation to metabolic functioning) is carried forward into adult life (Barker, 1992, 1998; Gluckman et al., 2008; Gluckman and Hanson, 2005). Few studies consider developmental programming hypotheses

* Corresponding authors. V. Glover is to be contacted at Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK. Fax: +44 207 594 2138. T.G. O'Connor, Department of Psychiatry, University of Rochester Medical Center, 300 Crittenden Blvd, Rochester, NY, 14642, USA. Fax: +1 585 276 2065.
E-mail addresses: v.glover@imperial.ac.uk (V. Glover), Tom_OConnor@URMC.Rochester.edu (T.G. O'Connor).

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for behavioral or psychological phenotypes (O'Donnell et al., 2009; Rutter et al., 2004). That may be changing, however, as human research begins to translate the experimental animal findings showing that prenatal stress is a central paradigm for demonstrating long-term effects of prenatal exposures on behavioral and biological development (Coe et al., 2003; Maccari et al., 2003; Weinstock, 2005). Abundant animal work using the prenatal stress paradigm points to glucocorticoid exposure as a leading candidate mechanism (Maccari et al., 2003; Matthews, 2000; Roughton et al., 1998; Seckl and Holmes, 2007; Weinstock, 2005). The specific mechanisms of action underlying the programming effect are unconfirmed but may include increased activation of the offspring HPA axis, alteration of glucocorticoid receptor sensitivity, and alteration of genetic expression in the stress response system. These findings underscore the need for further human work on glucocorticoid exposure on child outcomes. Particularly needed is research to examine whether key behavioral outcomes linked with prenatal stress and anxiety, such fear and emotionality (Bergman et al., 2007; Davis et al., 2007; O'Connor et al., 2002; Van den Bergh and Marcoen, 2004; see Talge et al., 2007 for a review), may be explained by glucocorticoid exposure.

In summary, the current study sought to add to, and connect, two lines of research emphasizing prenatal hormone exposure on offspring development: one focusing on prenatal testosterone and a second on glucocorticoids, which we index by cortisol. We include both measures study for two reasons. First, as noted, both testosterone and cortisol have been implicated in studies of human behavioral development, although the emphasis derived from different developmental models. A second reason is that, unlike in the adult, cortisol and testosterone are positively correlated in the fetus (Gitau et al., 2005; Sarkar et al., 2007b). Accordingly, before drawing conclusions about the effect of either on behavioral development, it is necessary to consider a possible joint activation and confound between the two.

We focus on fear reactivity because it is an index of a behavioral phenotype with a long history of developmental research (Rothbart et al., 2000) and because psychobiological work suggests it may be relevant to both cortisol and testosterone (Charney, 2004). By capitalizing on the leverage provided from amniotic fluid, the current study provides the most direct test in humans of a link between prenatal exposure to testosterone and cortisol and fear-related behavior in infancy.

Materials and methods

Mothers and babies were recruited as part of a prospective study on fetal hormone exposure and child development. Women were recruited sequentially from an amniocentesis clinic in a large urban maternity hospital between December 2001 and January 2005; women were referred to the clinic for karyotyping. Written informed consent was obtained in accordance with local research ethics committee requirement. All English-speaking mothers with full-term (≥37 weeks), healthy, and singleton infants, whose birth outcomes were known, were invited to return to the pediatric clinic in the hospital when the child was between 14 and 19 months old. Initially, 365 women were recruited at amniocentesis, of whom 109 were excluded because of known abnormalities, incomplete data on birth outcome or because the procedure was for non-routine amniocentesis. Of the 256 remaining mothers, we were unable to locate 71 and a further 60 did not wish to participate or could not attend the clinic (e.g., because of moving away from London), resulting in 125 children who were eligible and agreed to participate. For 17 children, it was not possible to complete the temperament assessment, primarily because of fatigue or time constraints in the lab visit, resulting in a sample of 108, for whom there were 108 cases with valid prenatal cortisol and 107 cases with valid prenatal testosterone. (There were two children living in non-native English-speaking homes; excluding these children did not alter the findings and so they were included given the non-language nature of the outcome.) Sample sizes in multivariate analyses differ slightly because of missing data on some covariates.

Amniotic fluid sampling and cortisol and testosterone analysis

During amniocentesis, an aliquot of up to 4 ml of amniotic fluid surplus to clinical requirement was drawn for the study and stored at −80 °C until assay. Time of collection, to the nearest 15 min, was recorded. Mean gestational age at the time of sampling was 17.2 weeks (median was 16 weeks; the range was 15–32, with 91% between 15 and 20 weeks). Total cortisol in amniotic fluid was assayed by radioimmunoassay (Coat-A-Count, DPC, Los Angeles, CA), cortisol having been extracted by dichloromethane and reconstituted prior to assay (Sarkar et al., 2007a). The intra- and inter-assay coefficients of variation for the amniotic fluid cortisol assay were 4.4% and 6.5%, respectively.

Total testosterone in amniotic fluid was measured by radioimmunoassay Coat-a-Count (DPC, Los Angeles, CA) after prior extraction by diethylether to minimize cross-reactivity. The intra- and inter-assay coefficients of variation of our testosterone assay procedures were 7.5% and 8.9%, respectively. Some questions have been raised about the reliability of the above method for analyzing amniotic fluid testosterone. Therefore, as a check on the reliability of that method, a random subset of the samples was analyzed by liquid chromatography/mass spectrometer (LCMS). Steroid hormone concentrations determined by an Agilent 1100 LCMS equipped with an electrospray ionization source and Chemstation software version A 10.02 were undertaken in the Assay Services Laboratories of the Wisconsin National Primate Research Center. All steroid hormones used as reference preparations were obtained from Steraloids (Newport, RI). The LCMS methods were validated using Federal Drug Administration protocols (May 2001) and adapted from those previously described (Abbott et al., 2008). Briefly, using positive ion identification for testosterone (m/z 289), the LCMS standard curves for testosterone used 0–4 ng/ml. Deuterated testosterone was added as internal standard to monitor recovery. The lower limit of quantitation was 0.02 ng/ml. The within-day coefficients of variation (for samples determined on the same day) were 2.7% and the between-day coefficients of was 5.1%. The correlation between LCMS testosterone data and radioimmunoassay testosterone data was $r(40) = 0.82$, $p < 0.001$.

Child temperament

The Laboratory Temperament Assessment Battery (Lab-TAB)—Locomotor Version (Goldsmith and Rothbart, 1999) was used to assess infant temperament. The Lab-TAB is a leading observational measure of childhood temperament, with considerable support for its validity and clinical and predictive value (Rothbart et al., 2000). It consists of 20 paradigms that are designed to elicit fear, anger/frustration, joy/pleasure, interest/persistence, and activity level. We used the unpredictable mechanical toy paradigm from the fear reactivity subscale and the paradigm for joy/pleasure.

During the unpredictable mechanical toy paradigm, the child sat at a table facing a puppet theater and a robotic dog was presented on the table when the child was calm and alert. Each trial lasted about 20 s and would consist of the dog barking walking towards the child as its eyes, mouth, and head moved. Three trials were presented to each infant unless the child was too distressed by an earlier trial to complete a subsequent trial. We report findings from the first trial (findings for the composite across trials – which carried forward scores for those unable to complete latter trials – were substantively identical to those from the first trial only). The episode was videotaped from behind the puppet theater and observational measures were later rated from videotape by a researcher blind to