Fetal Programming Effects of Testosterone on the Reward System and Behavioral Approach Tendencies in Humans

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Background: Sex differences are present in many neuropsychiatric conditions that affect emotion and approach-avoidance behavior. One potential mechanism underlying such observations is testosterone in early development. Although much is known about the effects of testosterone in adolescence and adulthood, little is known in humans about how testosterone in fetal development influences later neural sensitivity to valenced facial cues and approach-avoidance behavioral tendencies.

Methods: With functional magnetic resonance imaging we scanned 25 8–11-year-old children while viewing happy, fear, neutral, or scrambled faces. Fetal testosterone (FT) was measured via amniotic fluid sampled between 13 and 20 weeks gestation. Behavioral approach-avoidance tendencies were measured via parental report on the Sensitivity to Punishment and Sensitivity to Rewards questionnaire.

Results: Increasing FT predicted enhanced selectivity for positive compared with negatively valenced facial cues in reward-related regions such as caudate, putamen, and nucleus accumbens but not the amygdala. Statistical mediation analyses showed that increasing FT predicts increased behavioral approach tendencies by biasing caudate, putamen, and nucleus accumbens but not amygdala to be more responsive to positive compared with negatively valenced cues. In contrast, FT was not predictive of behavioral avoidance tendencies, either through direct or neurally mediated paths.

Conclusions: This work suggests that testosterone in humans acts as a fetal programming mechanism on the reward system and influences behavioral approach tendencies later in life. As a mechanism influencing atypical development, FT might be important across a range of neuropsychiatric conditions that asymmetrically affect the sexes, the reward system, emotion processing, and approach behavior.

Key Words: Approach behavior, emotion, fetal programming, fMRI, reward, testosterone

Many neuropsychiatric conditions affecting emotion processing and approach-avoidance behavioral tendencies (e.g., conduct disorder, psychopathy, attention-deficit/hyperactivity disorder, substance abuse, depression, bipolar disorder, cluster B personality disorders, intermittent explosive disorder, autism) (1) show sex differences in age of onset, risk, prevalence, and symptomatology (2–10). It is also noteworthy that developmental time periods for critical sex steroid surges co-occur with time periods where vulnerability for many of these conditions is elevated (e.g., adolescence), suggesting that mechanisms related to sexual differentiation might play a unique role (11). However, much more work is needed to understand how underlying developmental biological mechanisms related to sexual differentiation (e.g., sex chromosome or sex hormone effects) (12) might help to explain sex differences in these conditions. Unlike work in nonhuman species (13), it is not possible in humans to ethically and independently manipulate factors related to both sex chromosomes and sex hormones within a single study. Thus, in humans it is necessary to focus on each factor separately. In this study we focus on the role of testosterone during fetal development as one developmental biological mechanism that might influence phenotypic development in directions that might increase susceptibility for various neuropsychiatric conditions that asymmetrically affect the sexes.

In adolescence and adulthood, testosterone might increase susceptibility for such neuropsychiatric conditions by tipping the balance between approach and avoidance (14). For example, testosterone in adulthood decreases avoidance by attenuating unconscious fear-responses (15–17) and reducing sensitivity to punishment (18). Similar effects are found in adolescents (19). However, testosterone also increases sensitivity to approach-cues by enhancing attention to social threat (20–22), sensation seeking, motivation to act (23–25), and risk-taking and sensitivity to rewards (18,26). Functional magnetic resonance imaging (fMRI) studies in adolescence and adulthood mirror these effects. Testosterone reduces amygdala response to quick presentations of threat (27) but increases response to longer presentations of threat (28–31) and also increases ventral striatal (e.g., ventral caudate, nucleus accumbens, putamen) response to reward (32–34). Thus, in later life testosterone creates an imbalance between approach and avoidance. However, it is still unclear whether testosterone earlier in development plays a critical prior role in influencing approach-avoidance in brain and behavior.

Despite these later influences of testosterone, work in nonhuman species has shown that early developmental surges should be considered. Early androgens surges can exert "organizational" influence on brain development by laying down permanent cellular and molecular foundations that are necessary for later expression of sex differences (35–39). This idea is similar to the more general

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Received Mar 6, 2012; revised Apr 20, 2012; accepted May 9, 2012.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2012.05.027

idea of fetal programming, which suggests that early events in prenatal development permanently influence developmental paths and outcomes in later life (40). Steroid hormones are well-positioned as fetal programming mechanisms, because they exert substantial epigenetic influence on early brain development that lay the foundations for later interaction with the genome and environmental influences to create variation in neural and behavioral phenotypes (41–44). Thus, understanding the prior influence of testosterone on human brain development is important for understanding how it might program neural circuitry for biased responsiveness later in life and potentially lead individuals down multiple atypical developmental paths.

Here we present the first investigation in humans of how testosterone during fetal development predicts later neural response to valenced facial cues and individual differences in behavioral approach-avoidance tendencies. We tested a unique cohort of 25 boys (8–11 years) whose fetal testosterone (FT) was measured from amniotic fluid at 13–20 weeks gestation. Participants were scanned with fMRI while viewing negative (fear), positive (happy), neutral, or scrambled faces. This paradigm is known to elicit response in both amygdala and ventral striatum (45), which are also known to be influenced by testosterone (27–34). We predicted that increases in FT would predict decreased reactivity to negatively valenced facial cues and increased reactivity to positively valenced facial cues within amygdala and striatum. Furthermore, we predicted that such FT-mediated influence on neural response would predict behavioral approach-avoidance tendencies.

Methods and Materials

Participants

Participants were recruited as part of a longitudinal study of the effects of FT on cognitive, behavioral, and brain development. Initial screening consisted of reviewing medical records of patients who underwent amniocentesis in the Cambridgeshire (United Kingdom) region. Individuals were excluded if: 1) the amniocentesis revealed a chromosomal abnormality; 2) there was a twin pregnancy; 3) the pregnancy ended in termination or miscarriage; 4) relevant information was absent from the medical records; or 5) medical practitioners indicated it would be inappropriate to contact the family. Any child that presented with any developmental abnormalities postnatally was also excluded from testing. Twenty-eight right-handed typically developing boys were successfully scanned, but 3 were excluded for excessive motion, leaving 25 for the main analyses (mean age = 9.52, SD = .96, range: 8–11 years). Our use of a male-only sample was employed to eliminate potential nonhormonal confounds that systematically vary across male and female subjects (e.g., sex chromosome effects) (12). Informed consent was obtained from all the legal guardians of the participant in accordance with procedures approved by the local research ethics committee. This cohort has been previously reported in published works examining the relationship between FT and structural magnetic resonance imaging measures (38,46).

FT Collection and Measurement

Fetal testosterone was measured from amniotic fluid samples collected between 13 and 20 weeks of gestation (mean = .76 nmol/L, SD = .35 nmol/L, range = .25–1.70 nmol/L). This is within the 8–24-week period that is critical for human sexual differentiation (47). Six participants from the sample had missing data with regard to the exact time of amniocentesis. However, analysis of the remaining 19 participants (mean = 16.37, SD = 1.33, range = 14–19) showed that there was no relationship between gestational age at sampling and FT level ($r = .17$, $p = .47$), confirming prior work showing absence of a relationship (48). Thus, all 25 participants were included in the final analyses.

FT was assayed via radioimmunoassay. Amniotic fluid was extracted with diethyl ether, which was evaporated to dryness at room temperature, and the extracted material was re-dissolved in an assay buffer. Testosterone was assayed by the DPC 'Count-a-Coat' method (Diagnostic Product Corporation, Los Angeles, California), which uses an antibody to testosterone coated onto propylene tubes and a 125-I labeled testosterone analogue. The detection limit of the assay with the ether-extraction method is approximately .05 nmol/L. The coefficient of variation (CV) for between-batch imprecision is 19% at a concentration of .8 nmol/L and 9.5% at a concentration of 7.3 nmol/L. The CVs for within-batch imprecision are 15% at a concentration of .3 nmol/L and 5.9% at a concentration of 2.5 nmol/L. This method measures total extractable testosterone.

Task Design and Behavioral Measures

Participants were scanned while viewing fear, happy, neutral, and scrambled faces taken from the standard Karolinska Directed Emotional Faces set (49). Specific stimuli were chosen from the Karolinska Directed Emotional Faces on the basis of unanimous ratings of the target expression from five independent judges.

For each trial a face was presented on the screen for 2000 msec, followed by a central crosshair for 750 msec, followed by an intertrial interval of 312 msec before the onset of the next trial. Conditions were presented in separate blocks, with 8 trials/block. Each block lasted 24.5 sec and was repeated four times in pseudorandom order. Throughout the experiment, participants were instructed to press a button with their right index finger whenever a face was presented. Stimulus presentation was implemented with DMDX software (http://www.u.arizona.edu/~kforster/dmdx/dmdx.htm), and stimulus presentation was synchronized with the onset of the functional run to ensure accuracy of event timing.

Individual differences in behavioral approach-avoidance tendencies were assessed by caregiver report on a modified version of the Sensitivity to Punishment and Sensitivity to Rewards questionnaire (50). Previous factor analyses suggest that the Sensitivity to Punishment and Sensitivity to Rewards questionnaire can be split into 4 subscales: Punishment, Impulsivity/Fun-Seeking, Drive, and Reward Responsivity. The Punishment scale was used as our measure of avoidance tendencies and was termed "BIS" after Gray's "behavioral inhibition system" (51). For behavioral approach tendencies, we created a summary score by summing across all items on the other three scales (Impulsivity/Fun-Seeking, Drive, and Reward Responsivity). We term this summary score "BAS" after Gray's "behavioral activation system" or "behavioral approach system" (51).

fMRI Data Acquisition

All imaging took place at the Wolfson Brain Imaging Centre at Addenbrooke's Hospital, Cambridge, United Kingdom, on a Siemens Tim Trio 3 Tesla magnet (Siemens Medical Solutions, AG, Erlangen, Germany). Our functional imaging run consisted of 200 whole-brain functional T2*-weighted echoplanar images (slice thickness, 3 mm; .75 mm skip; 32 axial slices; repetition time, 2000 msec; echo time, 30 msec; flip angle, 90°; matrix, 64 × 64; field of view, 192 mm; interleaved slice acquisition). The first six timepoints of the run were discarded to allow for T2 stabilization effects. In addition, a high-resolution T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient-echo (MP-RAGE) structural image was acquired for registration purposes (slice thick-