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INVITED REVIEW

Prenatal and postnatal hormone effects on the human brain and cognition

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Abstract This review examines the role of hormones in the development of social and nonsocial cognition and the brain. Research findings from human studies designed to elucidate the effects of both prenatal and postnatal exposure to hormones in children and young adults are summarized. Effects are found to be both time and dose dependent, with exposure to abnormal hormone levels having a limited impact outside the "critical window" in development. Particular attention is given to the role of prenatal hormone exposure, which appears to be vital for early organization of the brain. In later life, measurements of circulating hormone levels and the administration of testosterone and oxytocin are found to predict behavior, but the effect is thought to be one of "activation" or "fine-tuning" of the early organization of the brain. Possible directions for valuable future research are discussed.

Keywords Prenatal testosterone · Postnatal testosterone · Testosterone administration · Oxytocin · Sex differences · Amniotic fluid · Amniocentesis · Puberty

Hormones are some of the most important chemicals that our bodies manufacture. They are the messengers that we use to regulate and control virtually all our physiological processes, from metabolism (e.g., adrenaline, insulin) to activation of the immune system (e.g., erythropoietin) and the regulation of mood (e.g., oxytocin). They are also essential in the processes of reproduction (e.g., testosterone, estrogen), and growth and development (e.g., somatotropin) [90]. In this review, we discuss whether hormones are also related to our behavioral development.

Animal studies on the effects of hormones have been conducted for over half a century and provide some of the clearest evidence for the role of various hormones in our bodies. In particular, manipulation of glands producing particular hormones can have startling effects on physical development as well as later behavior (e.g., [34, 40, 60, 61, 73]). Mammals have been widely studied, with castration (and subsequent reduction in the availability of gonadal hormones) a common early experiment. These experiments show that hormones are essential to the sexual differentiation of both the body and the brain (see Collaer and Hines [40] for a review). It has been recognized for a long time that castration of males during neonatal or prenatal life prevents the development of masculine genitalia, while administration of androgens to females masculinizes their genitalia [81]. Castrated males also usually show feminized neural development, cognition, and behavior; while females treated with androgen show masculinized neural development, cognition, and behavior. Similar experiments have been conducted in a wide range of mammals, comparing castrated males, normal males, normal females, and females treated with androgens on a range of sexually dimorphic features consistently demonstrating the importance of sex steroid hormones (testosterone in particular) in the development of the brain and behavior [6, 30, 61, 98, 139].

While the effects of testosterone on nonhuman mammal sexual behavior have been extensively studied, there is now increasing evidence that this and other hormones also have a substantial effect on aspects of human social and emotional behavior [39, 71]. This review aims to examine these findings and, in particular, to present a series of longitudinal studies designed to elucidate the behavioral effects of both prenatal and postnatal testosterone exposure in children and young adults.

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Timing and critical periods

The timing of hormonal effects is crucial when studying lasting effects on development. There are generally thought to be two types of hormonal effects: organizational and activational [109]. Organizational effects are most likely to occur during early development when most neural structures are being established, producing permanent changes in the brain [109], whereas activational effects are short term and are dependent on current hormone levels. It is widely thought that organizational effects are maximal during certain critical periods of development. These are hypothetical windows of time in which a tissue can be formed [71]. Outside the sensitive period, the effect of the hormone will be limited, protecting the animal from disruptive influences. This means, for example, that circulating sex hormones necessary for adult sexual functioning do not cause unwanted alterations to tissues, even though the same hormones might have been essential in laying down cellular organization during the initial development of those tissues.

Animal research has indicated that the critical period for sexual differentiation of the brain occurs when differences in serum testosterone are highest between sexes [40]. Therefore, it is likely that this is an important period for sexual differentiation of the human brain as well. It is difficult to get accurate measurements of hormone levels for humans, but studies that have sampled fetal serum, plasma, and amniotic fluid during pregnancy have indicated that for typical human males, there is a surge in fetal testosterone (FT) levels between weeks 8 and 24 of gestation, peaking around week 16 [2, 36, 115, 116, 126]. During this period, male fetuses produce more than 2.5 times the levels observed in females [20]. There is then a decline to barely detectable levels from the end of this period until birth. As a result, it is expected that the most significant effects of FT on development are likely to occur within this window. For typical human females, levels are generally very low throughout pregnancy and childhood [71].

In addition to the fetal surge, two other periods of elevated testosterone have been observed in typical males. The first takes place shortly after birth and reaches a peak when the child reaches approximately 3–4 months [126], and the second occurs around puberty. Figure 1 shows the typical sex differences in circulating testosterone levels during the prenatal and neonatal period.

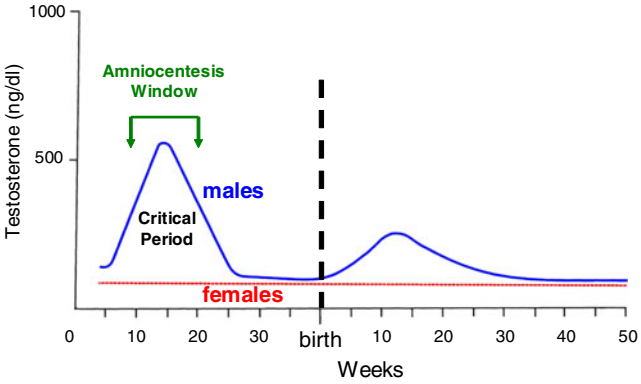

Fig. 1 Circulating levels of testosterone in the human fetus and neonate. Males (*blue line*) have been shown to have higher levels of testosterone than females (*red dashed line*), particularly from about week 8 to 24 of gestation and week 2 to 26 of postnatal life. (Figure adapted from Hines [71]. *Brain gender*. New York, NY: Oxford University Press, Inc.)

Prenatal hormone effects in humans

Studies in clinical conditions

Some naturally occurring clinical conditions render the human hormone environment abnormal. As we consider artificial manipulation of the hormone environment during critical periods of development to be unethical, such conditions are a natural starting point for evaluating the impact of androgens and other hormones on development.

One such condition is congenital adrenal hyperplasia (CAH), a genetic disorder which causes excess adrenal production of androgen hormones (including testosterone and other hormones thought to be responsible for the development of masculinizing features) beginning prenatally in both males and females [103].

Studies of individuals with CAH have generally found that girls with the condition show masculinization of behavioral performance in activities such as spatial orientation, visualization, targeting, personality, cognitive abilities, and sexuality [64, 74, 114]. Several research groups have reported that girls with CAH show increased male-typical toy, playmate, and activity preferences [49, 70, 71, 106]. The determining role of prenatal steroids in sex-role identity appears to be supported by studies of females with CAH who demonstrate a masculine bias on various personality inventories (e.g., Detachment and Indirect Aggression Scales, Aggression and Stress Reaction Scales, Reinish's Aggression Inventory) [40].

While CAH provides an opportunity to investigate the effects of additional androgen exposure, the relatively rare occurrence of CAH makes it difficult to obtain large-enough groups for generalization of research findings to the wider population. Researchers have also suggested that CAH-related disease characteristics, rather than prenatal androgen exposure, could be responsible for the atypical cognitive profiles observed in this population [51, 112].

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women and affects an estimated 1 in 15 women worldwide. It is a heterogeneous disorder generally characterized by disruption of the ovulation cycle, a number of small cysts around the edge of their ovaries (polycystic ovaries), and excessive production and/or secretion of androgens (masculinizing hormones) referred to as

"hyperandrogenism" [104]. A study of children of women with PCOS found that daughters of these women showed lower Empathy Quotient (EQ) scores, a measure where girls generally show higher scores than boys [105]. In the same study, daughters of women with PCOS also showed higher Systemizing Quotient (SQ) scores, a measure where boys generally score higher than girls. These findings are consistent with the idea that PCOS increases androgen exposure in the womb and that this increased exposure leads to more masculinized behavior in later life.

Studies using amniotic fluid measurements

Amniocentesis is the process of extracting a sample of amniotic fluid during the second trimester of pregnancy to detect clinical abnormalities in mothers thought to be at risk. Amniocentesis is typically performed during a relatively narrow time window which is thought to coincide with the hypothesized critical period for human sexual differentiation (between approximately weeks 8 and 24 of gestation, see Fig. 1) [71]. Samples taken this way indicate that both male and female human fetuses produce testosterone, with male fetuses producing on average 2.5 times the levels observed in females; see Fig. 2.

Males are exposed to testosterone from the fetal adrenals and testes. The female fetus is also exposed to androgens, but at much lower levels. In early prenatal life, this testosterone enters the amniotic fluid via diffusion through the fetal skin and later enters the fluid via fetal urination [118]. Testosterone levels are also affected by other processes—the underproduction of aromatase may result in higher FT levels by impairing conversion of testosterone to estrogen [1]. Similarly, dihydrotestosterone is produced from testosterone and may be a stronger activator of the androgen receptor than testosterone itself [90]. While these processes limit the conclusions we can draw from a snapshot measurement of FT level in the amniotic fluid, it is a useful starting point from which to develop our understanding.

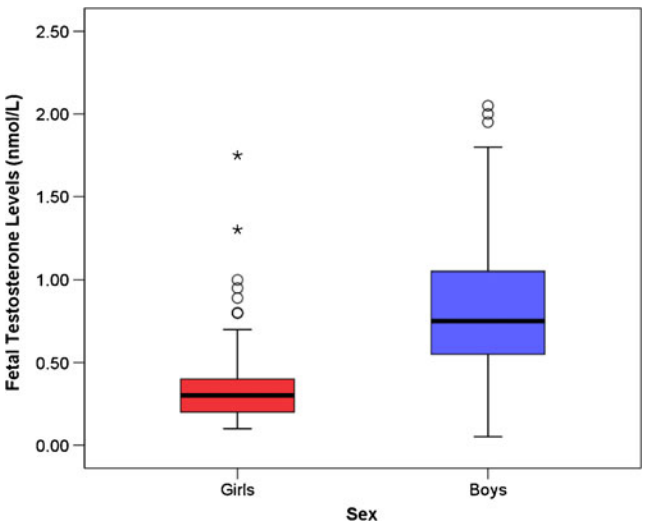

Fig. 2 Sex differences in amniotic fluid measurements. (Data obtained from the Cambridge Child Development Project). *Circles* indicate a small deviation from the mean. *Stars* indicate a large deviation from the mean

A number of studies have linked elevated levels of FT in the amniotic fluid with the masculinization of certain behaviors, beginning shortly after birth. In particular, the Cambridge Child Development Project is an ongoing longitudinal study investigating the relationship between prenatal hormone levels and the development of later behaviors [15, 85]. Mothers of participating children had all undergone amniocentesis for clinical reasons. To date, these children have been tested postnatally at several time points.

Eye contact at 12 months

Reduced eye contact is a characteristic common in children with autism [96, 129]. The first study aimed to measure FT and estradiol levels in relation to eye contact in a sample of 70 typically developing 12-month-old children [96]. Frequency and duration of eye contact were measured using videotaped sessions. Sex differences were found, with girls making significantly more eye contact than boys. The amount of eye contact varied with FT levels when the sexes were combined and also within the boys' group [96]. No relationships were observed between the outcome and estradiol levels. Results were taken to indicate that FT may play a role in shaping the neural mechanisms underlying social development [96].

Vocabulary size at 18–24 months

Another study of 87 children focused on the relationship between vocabulary size in relation to FT and estradiol levels from amniocentesis. Vocabulary size was measured at both 18 and 24 months of age using the Communicative Development Inventory, a self-administered checklist of words for parents to complete [63]. Girls were found to have significantly larger vocabularies than boys at both time points [97], and results showed that levels of FT inversely predicted the rate of vocabulary development [97].

Autistic traits in toddlers at 18–24 months

Autism spectrum conditions (ASCs) are a group of related conditions characterized by impairments in reciprocal social interaction and communication, alongside strongly repetitive behaviors and unusually narrow interests [5]. It has been well documented that autism is much more prevalent in males than females [32, 58], so the possibility that androgens may have a role to play in the etiology of these conditions was explored.

Studies have examined the effects of FT on the later development of autism and autistic traits. In the first of these studies, autistic traits were measured using the Quantitative Checklist for Autism in Toddlers (Q-CHAT) [4]. This questionnaire has been used to measure autistic traits elsewhere and has been validated by following children throughout their early development (Fig. 3 shows an example question).

The Q-CHAT questionnaire was administered to mothers who had also undergone amniocentesis, providing measurements of FT level and fetal estradiol (FE)—a second hormone which forms prenatally from testosterone and is considered to be the most biologically active estrogen [40]. Samples of postnatal testosterone (PT) levels were also taken from saliva at 3–4 months of age in a small sample of these children. The study revealed a significant sex difference in autistic traits, with boys scoring higher (indicating more autistic traits) than girls. Q-CHAT scores were predicted by FT levels only, with both sex and the FT/sex interaction excluded from the model [13].

The relationship between FT and Q-CHAT score was also visible within the subset of children who participated in the follow-up study measuring PT levels at 3–4 months. However, no relationships between FE, PT levels, and Q-CHAT scores were observed. In addition, FE and PT levels showed no sex differences or relationships with FT levels [7].

Use of mental and affective language at 4 years

The Cambridge Child Development study has also completed much longer-term studies, with participants recruited at amniocentesis being followed up 4–5 years after birth. This has provided the opportunity to establish a much greater understanding of how FT levels could affect behavioral development in later life.

Thirty-eight children completed a "moving geometric shapes" task at age 4. The children were asked to describe cartoons with two moving triangles whose interaction with each other suggested social relationships and psychological motivations [87]. Sex differences were observed, with girls using more mental and affective state terms to describe the cartoons compared to boys; however, no relationships between FT levels and mental or affective state terms were observed. Girls were found to use more intentional propositions than males, and a negative relationship between FT levels and frequency of intentional propositions was observed when the sexes were combined and in boys. Boys used more neutral propositions than females. FT was related to the frequency of neutral propositions when the sexes were combined.

Fig. 3 Example items from the Q-CHAT

Social relationships and narrow interests at age 4 years

A separate follow-up study at 4 years of age utilized the Children's Communication Checklist—a questionnaire designed to screen for communication difficulties in children 4–16 years of age [24]. A quality of social relationships subscale demonstrated an association between higher FT levels and poorer quality of social relationships for both sexes combined (but not within each sex). A lack of significant correlations within each sex was thought to be a result of the small sample size (n=58).

Levels of FT were also associated with a subscale for narrower interests when the sexes were combined and also within boys [86]. This within-sex result is interesting since it suggests that this subscale of the measurement is sensitive to even moderate changes in FT level. Sex differences were also reported, with males scoring higher (i.e., having more narrow interests) than females [86].

Gender-typical play at 6–9 years

At 6–9 years of age, the children from the Cambridge Child Development Project were followed up using the Pre-School Activities Inventory (PSAI). This is a standardized questionnaire measure of gender-typical play in both boys and girls. The PSAI includes 24 items and is completed by a parent to describe the child's behavior. Higher scores reflect more male-typical behavior, and females with CAH obtain elevated (more male-typical) scores in the PSAI [72], suggesting sensitivity to the effects of prenatal androgen exposure. A significant relationship was found between FT levels and sexually differentiated play behavior in both girls and boys [9].

Gender-role behavior at 6–9 years

The Bem Sex Role Inventory (BSRI) is a questionnaire developed to measure feminine and masculine personality traits on the basis of cultural definitions of sex-typed social desirability [21]. This is a 60-item (20 feminine, 20 masculine, and 20 non-gender-related items) questionnaire. Examination of scores in this measure indicated that higher FT levels are associated with higher masculinity scores in the

BSRI when boys and girls were examined together and when girls were examined alone. No relationships were found between FT levels and scores in the femininity scale. Within-sex results suggest that girls exposed to higher testosterone levels in utero are perceived as exhibiting more masculinized behavior [7].

Mind reading at 6–9 years

Mind reading is the ability to put oneself into the mind of another person and infer what the person is thinking or feeling. It is also referred to as theory of mind [91] or mentalizing [55]. One method of measuring an individual's capability for mentalizing is the child version of the "Reading the Mind in the Eyes" test. This measure consists of 28 pictures from the eye region of the face, with each depicting a mental state—some including subtle emotions [16]. Figure 4 shows an example item, with four emotions for the participant to choose from (the correct answer is "a bit worried").

Results from this study revealed a significant negative correlation with FT—with higher levels predicting lower mind reading capability. Within-sex analyses revealed a significant negative correlation between FT and the eyes test within both the boys' and girls' groups [33]. The significance within each sex points to a much more sensitive dependency on FT level than in the entire population, where boys have much higher levels of FT.

Empathy and systemizing at 6–9 years

Empathy has been described as the drive to identify another person's emotions and thoughts and to respond to these appropriately [14]. This is an aspect of social interaction where there is usually a strong advantage for females. Sex differences in the precursors of empathy are seen from birth, with female babies showing a stronger preference for looking at social stimuli (faces) 24 h after birth [41] and more eye contact at 12 months of age [96]. Girls also tend to show more comforting, sad expressions or sympathetic vocalizations than boys when witnessing another's distress as early as 1 year of age [76].

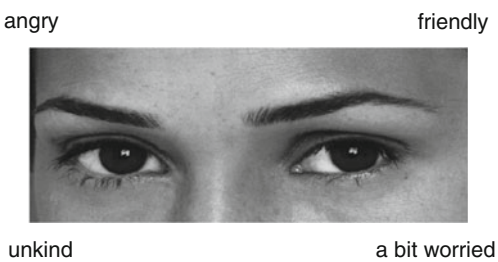

Fig. 4 Example item from the children's Reading the Mind in the Eyes Test. The child is asked to indicate which of the four choices best describes what the person in the picture is feeling

Systemizing has been defined as the drive to analyze, explore, and construct a system [14]. Systemizing allows one to predict the behavior of a system and to control it. A system is defined as something that takes inputs, which can then be operated on in *variable* ways, to deliver *different* outputs in a rule-governed way. A growing body of evidence suggests that, on average, males spontaneously systemize to a greater degree than females. Boys, on average, engage in more mechanical and constructional play than girls [22, 92]. This sex difference in toy choice has been observed in humans as early as the first year of life [125], as well as in nonhuman primates [3], suggesting the possibility of a biological basis for these preferences. Boys are better than girls at using directional cues in map reading and map making [19, 56, 82]. They are also more accurate on the Mental Rotation Test [80, 100] and the Rod and Frame Test [23, 141]. All of these can be seen as involving systemizing since they involve relating input to output via a lawful operation.

The Cambridge Child Development study has developed parental questionnaires to assess a child's capability in the above dimensions: the Empathy Quotient (EQ-C) and Systemizing Quotient (SQ-C). These questionnaires are based on similar versions for adolescents and adults, which have consistently identified significant differences between males and females [8, 138]. Although limited by the questionnaire format, these measures have the advantage that parents observe their child's behavior in a wide number of contexts.

Girls generally scored higher than boys on the EQ-C at ages 6–8 years. When scores were compared with prenatal measurements of FT, a significant negative correlation between FT levels and EQ-C score was observed when the sexes were combined and also within boys [33].

Results for the SQ-C showed that boys scored significantly higher than girls on this scale. A significant positive association was found between SQ-C and FT levels when boys and girls were examined together. When sexes were examined together, the only significant predictor was FT. Sex was not included in the final regression model, suggesting that FT levels could play a greater role than the child's sex in terms of differences in systemizing preference [10].

The dimensions of Empathizing and Systemizing are also useful to our understanding of autism. Administration of a wide variety of tasks measuring the ability to empathize has demonstrated that individuals with an ASC are much weaker in this area. Conversely, there is some evidence that some individuals with an ASC are better at tasks that involve systemizing.

Autistic traits at 6–9 years of age

In order to further evaluate the potential effects of prenatal exposure to testosterone on the development of autistic behaviors in later life, FT measurements were directly

evaluated against a child's score on the Childhood Autism Spectrum Test (CAST) [123, 140] and the Child Autism Spectrum Quotient (AQ-Child) [12]. The CAST is a validated and widely used autism screening measure used to detect who is at risk for ASC. The AQ-Child is a measure that quantifies autistic traits and has been used widely in research.

FT levels were positively associated with higher scores (indicating greater number of autistic traits) on both the CAST and the AQ-Child. For the AQ-Child, this relationship was seen within both the male and female groups as well as when the sexes were combined, suggesting that this is an effect of FT rather than an effect of sex. The relationship between CAST scores and FT was also seen within boys, but not girls [11].

Summary of the Cambridge Child Development Project

Table 1 describes the measures used in the Cambridge Child Development Project to identify sex differences in behavior and the links with FT for boys and girls together. For each measure, we describe the direction of the sex differences (if present). The final columns indicate whether FT levels (independent of sex) were a significant predictor in the associated regression analyses.

FT and the brain

Results from the above studies suggest that higher prenatal hormone levels contribute to greater masculinization of behavior. In order to understand some of the mechanisms by which this could take place, we have recently extended our investigation into how FT may affect brain development.

Increased FT levels have been shown to affect brain morphology, showing a significant relationship with increased rightward asymmetry (e.g., right > left) of a posterior subsection of the callosum [35]. FT also influences later cortical gray matter volume, which has been observed to be sexually dimorphic [94]. Higher FT predicted increased gray matter in the right temporo-parietal junction, and this brain region shows a male > female pattern of sexual dimorphism. The right temporo-parietal junction is one region that is associated with tasks requiring one to think about other people's thoughts and mental states [121], suggesting a link between FT exposure and the neural development of mentalizing. Similarly, the gray matter in the planum temporale and posterior lateral orbitofrontal cortex is inversely related to FT levels and also show a female > male pattern of sexual dimorphism. Thus, FT predicts development of gray matter in directions that are congruent with observed sexual dimorphism and is indicative of the organizational nature of its influence on sexually dimorphic brain development; see Fig. 5.

Recent studies on functional brain response have also indicated that higher levels of FT predict enhanced sensitivity to positive (happy faces) compared to negatively valenced information (fear faces) in reward-related structures within the striatum. Furthermore, the effect FT has on influencing sensitivity to positive over negatively valenced information mediates the relationship FT has on predicting later behavioral approach tendencies (e.g., When my child sees an opportunity for something, he/she gets excited right away) [93].

Table 1 Cambridge Child Development Project results

Characteristic	Measure	Child age	Sex difference	FT sig. predictor
Eye contact	Frequency	12 months	Yes (F > M)	Yes
Vocabulary size	Communicative Development Inventory	18–24 months	Yes (F > M)	Yes
Mental and affective language	Intentional Propositions	4 years	Yes (F > M)	Yes
Restricted interests	Children's Communication Checklist	4 years	Yes (F < M)	Yes
Social relationships	Children's Communication Checklist	4 years	Yes (F > M)	Yes
Gender-typical play	Pre-School Activities Inventory	6–9 years	Yes (F < M)	Yes
Gender-role behavior	Bem Sex Role Inventory Femininity Total	6–9 years	Yes (F > M)	–[a]
	Bem Sex Role Inventory Masculinity Total		Yes (F < M)	Yes
Mind Reading	Reading the Mind in the Eyes	6–9 years	No	Yes
Empathy	Empathy Quotient	6–9 years	Yes (F > M)	No
Systemizing	Systemizing Quotient	6–9 years	Yes (F < M)	Yes
Autistic traits	Q-CHAT	18–24 months	Yes (F < M)	Yes
Autistic traits	AQ-Child	6–10 years	Yes (F < M)	Yes
Autistic traits	CAST	6–10 years	Yes (F < M)	Yes

[a] A regression analysis was not conducted

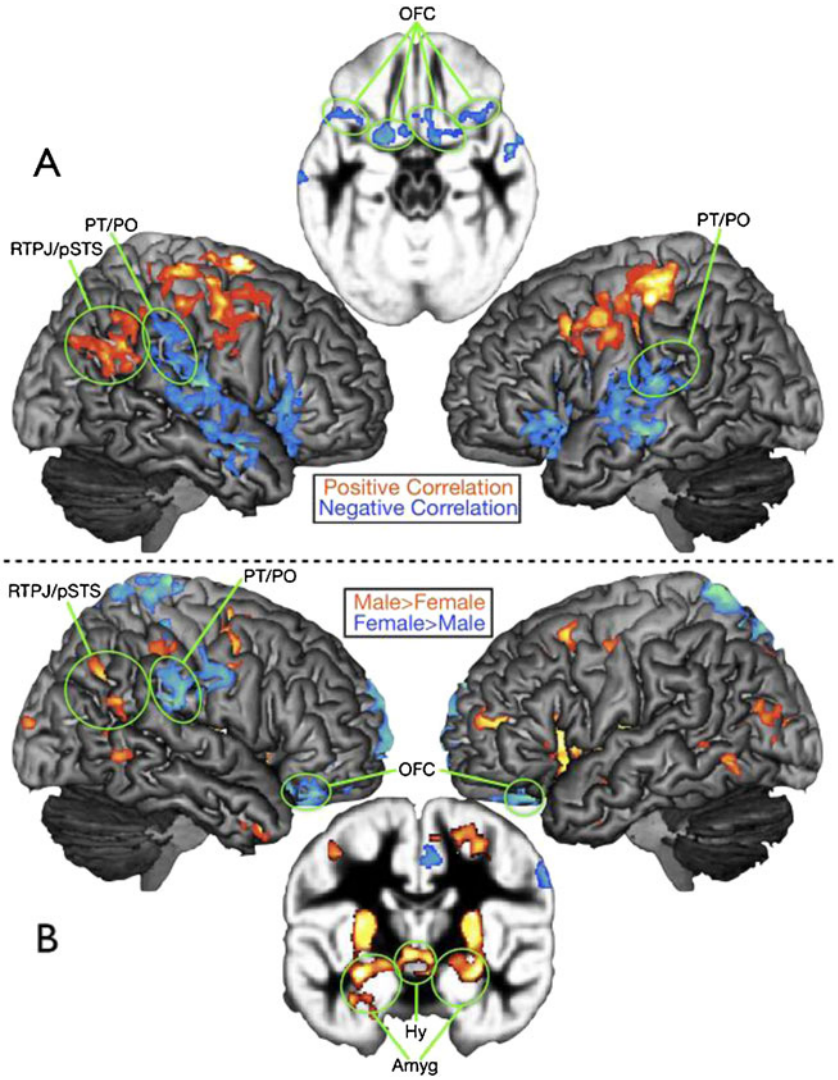

Fig. 5 FT correlations with gray matter volume. **a** Areas where FT predicts local gray matter volume. *Red/orange voxels* denote positive correlations; *blue voxels* denote negative correlations. **b** Areas of sexual dimorphism in local GM volume. *Red/orange voxels* denote a male > female pattern; *blue voxels* denote a female > male pattern. (Figure from Lombardo et al., [94])

Limitations of measuring prenatal exposure to hormones in amniotic fluid

The findings presented in Table 1 make use of testosterone levels in amniotic fluid (via amniocentesis). The benefit of this method is that it provides a sample which is close to the fetus and which is collected as part of normal clinical practice for mothers thought to be at risk of complications during pregnancy or birth. Amniocentesis is generally also conducted in a fairly narrow time window, aiding repeatability of measurements. Ideally, it would be best to make direct measurements of testosterone at regular intervals throughout gestation and into postnatal life. Even for amniocentesis, it is not currently possible to obtain repeated samples of FT because the procedure carries a risk of causing miscarriage (of about 1 %) [42, 120]. It is also known that hormones fluctuate during the day and between days, even in fetuses [124, 137].

Given the estimated timeline for testosterone secretion, the most promising time to measure FT is probably at prenatal weeks 8 to 24 [126], but this is still a relatively wide range. Research on nonhuman primates has also shown that androgens masculinize different behaviors at different times during gestation, suggesting that different behaviors may also have different sensitive periods for development [60].

For all these reasons, the inferences we can therefore draw about the single measurement of FT are necessarily limited. At the same time, a significant correlation between amniotic FT and a behavior should represent a conservative estimate of the potential effect of FT exposure on that behavior.

Human behavior is complex, and biological, social, or cultural factors are continuously interacting, making it challenging to investigate the causes of behavior. To the extent that social factors have been considered within the experiments presented so far, these have been restricted to certain

demographic variables (such as maternal age, parental education, and number of siblings), and behaviors and traits are likely to be influenced by a range of social factors that have not been measured in these studies.

Postnatal hormone effects in humans

Studies of current (activational) hormones

Studies of postnatal hormone exposure have examined the effects of current (or activational) hormones. The most obvious example of postnatal hormone exposure is during puberty—a major period of hormonal, physical, and behavioral change and development.

Studies in nonhuman mammals have investigated whether changes during puberty indicate a critical period for the effects of steroid hormones. One study showed that gonadectomy in male ferrets before puberty but after the early critical period did not affect sexual development when these animals were treated with testosterone in adulthood [17]. A study in rats showed that early steroid hormone deprivation resulted in systemic reduction in sensitivity to later androgen effects [59]. More recent findings suggest that steroid hormones during puberty have an activational affect on brain development [122]. These results indicate that although the critical window during perinatal development is vitally important for early sexual differentiation of the brain, the pubertal period also plays a large role in "fine-tuning" the organizational effects of steroid hormones [119].

In the area of physical development, it is well known that during puberty changes in adrenal androgens, rapid growth in body size, changes in fat composition, and the development of secondary sex characteristics occur [54]. In contrast, the studies examining the relationships between puberty, hormone changes, and the effects on social and emotional behavior have been relatively few. This is because the onset of puberty varies greatly between individuals as well as between sexes, so recruitment of appropriate age groups can be difficult. In addition, there is little research on this age group due to the discomfort and embarrassment that may occur when trying to obtain reliable and accurate information on sexual maturation. Other studies have relied on parental or self-report measures of puberty, which can also have difficulties [107]. It is also very difficult to disentangle the many physical aspects of maturation from the co-occurring changes in social and cultural status that are associated with this age group.

Much of what is known about adolescent development in humans comes from studies that do not specifically include biological measures of pubertal development (such as hormone levels). For example, there is evidence suggesting that brain regions such as the rostral prefrontal cortex which are involved in certain executive functions are still developing during adolescence [48]. A study that used a narrow age range and measures of pubertal development showed a positive correlation between pubertal development and an increased tendency towards sensation seeking [99]. The increase in sensation seeking during puberty may relate to the increase in risk taking observed in adolescents, which seems to decline in adulthood [145]. However, how the development of these systems might be related to the effect of puberty or changes in steroid hormones is not known.

Efforts have also been made to examine the links between both prenatal as well as activational hormone effects on aggressive behavior in same-sex and opposite-sex twins, with the assumption that girls from pairs of opposite-sex twins are exposed to higher levels of prenatal testosterone compared to same-sex twin girls. The researchers hoped to control for postnatal environmental effects by comparing data with similar measurements of same-sex female twins. In this study, the Dutch translation of the Reinisch Aggression Inventory (RAI) [113] and the Dutch translation of a modified version of the Olweus Multifaceted Aggression Inventory (OMAI) [52] were used to measure aggression in 74 opposite-sex and 55 same-sex 13-year-old twin pairs. Opposite-sex twin girls scored in the masculine direction on the withdrawal and verbal aggression subscales of the RAI, whereas no differences were observed between same-sex and opposite-sex twin girls on the OMAI. These differences may have existed because the RAI measures how prone an individual is to aggressive behavior, whereas the OMAI focuses on overt aggressive behavior [38]. The activational effects of testosterone were assessed using salivary testosterone measures in addition to a measure of pubertal status using the Tanner drawings [130]. Although there was some evidence of associations between free testosterone levels and personality traits (such as aggressive impulses and boredom susceptibility in boys, and experience seeking and extraversion in girls), the authors concluded that at this age, no clear associations between circulating testosterone levels and behavioral traits were apparent [38].

More recently, sex differences were observed in the relationship between circulating testosterone levels using bloodspot samples and thickness in areas of the brain which are associated with high androgen receptor density (including the left inferior parietal lobule, middle temporal gyrus, calcarine sulcus, and right lingual gyrus) [29]. These findings provide new evidence for the role of testosterone in pubertal structural brain development and sexual differentiation; however, further work is needed to ascertain how these changes may relate to social, cognitive, and emotional development.

Studies of testosterone administration

The majority of findings discussed so far have relied on observations in clinical conditions characterized by atypical

exposure to hormones or by obtaining samples of amniotic fluid, blood, or saliva to measure hormone levels and relating these to measurements of interest. In some cases, it is also possible to study the effects of directly altering circulating hormone levels (though prenatal manipulation of hormone levels is considered too dangerous and unethical). Recent studies in adult women have used a sublingual administration of testosterone, leading to a short-term large increase in circulating testosterone. Using this method, a series of studies have examined the effects of a single dose of testosterone versus placebo on social and emotional behavior (see Bos et al. [27] for a review).

Administration studies have shown that testosterone decreases theory of mind and facial emotion recognition in these women. Using the Reading the Mind in the Eyes test, a measure examining subtle emotion and mental states from pictures of the eye region, testosterone administration led to lower scores compared to placebo [134]. Interestingly, the 2D:4D digit length ratios (thought to be a proxy for prenatal hormone exposure) of the women tested in this study predicted approximately 50 % of the variance in the effect of testosterone on task performance. The authors suggest that the testosterone administration effect may be primed by prenatal exposure to testosterone [134].

Testosterone administration has also been shown to decrease recognition of angry expressions, and the authors hypothesize that testosterone may reduce the recognition of social threat, which may point towards a role for testosterone in social aggression [133]. Angry faces may be an implicit signal of threat or competition, and testosterone administration has also been shown to increase gaze to the eye region of threatening faces that are viewed unconsciously, suggesting a role for testosterone in implicit social dominance [131]. Testosterone has also been shown to reduce empathic facial imitation [68]. In a functional MRI (fMRI) study, testosterone administration activated areas such as the orbitofrontal cortex and amygdala (both considered to be emotion processing regions) when looking at angry-versus-happy facial expressions, again suggesting a role for testosterone in social threat [69]. A recent fMRI study also suggests that administration of testosterone alters functional connectivity between brain regions when looking at social stimuli. Testosterone (vs. placebo) decreases connectivity between the amygdala and orbitofrontal cortex (OFC) [26], and amygdala activation shifts away from the OFC towards the thalamus [136].

Testosterone is also related to trust. Administration of testosterone is related to rating pictures as being less trustworthy compared to placebo, even when baseline testosterone levels do not differ [28]. Administration of testosterone increases the responsiveness of the amygdala to untrustworthy faces, perhaps due to heightened social vigilance [26].

Testosterone decreases the amount of collaboration between two participants by increasing the egocentricity of the individual's choices [143] and decreases generosity [144]. However, another study found that testosterone administration increases social cooperation in individuals with low levels of prenatal testosterone exposure (measured using 2D:4D ratio) [132], which provides some evidence that responses following testosterone administration may be in part dependent on early organizational effects. Following testosterone (vs. placebo) administration, women have increased activation in the thalamo-cingulate region, insula, and the cerebellum in response to infant crying, indicating testosterone may have a role in modulating parental care [25].

Testosterone also affects responsivity to reward. Using the Iowa gambling task, women show an increase in risk taking after testosterone administration [135]. Using a monetary incentive delay task, testosterone administration increases ventral striatum activation, associated with reward anticipation, in individuals with low appetitive motivation (behavior directed toward goals that are usually associated with reward processes) [67].

Participants who *believe* that they received testosterone, regardless of whether they actually received it or not, behave more unfairly than those who believed that they were treated with placebo. In fact, testosterone administration increases the frequency of fair bargaining [50].

Although these studies provide interesting and novel evidence for testosterone administration effects, the sample sizes are small and further replication of the results is needed. These studies also include mainly females, and while they do control for the phase of the menstrual cycle, which itself predicts emotion recognition [43] and brain function (e.g., amygdala response) [44], many of the women are also using oral contraceptives which suppress ovarian hormone production [53]. The effects of how all these factors interact and the effects of social and emotional behavior need further investigation.

Studies of oxytocin administration

Oxytocin is another hormone that has been shown to be essential to our social functioning. Interestingly, research examining the administration of oxytocin has shown seemingly "opposite" results to those found for testosterone when examining its effect on aspects of human social behavior.

Oxytocin production is unique to mammals, and a great deal of research has investigated the critical role it plays in the social behavior of nonhuman mammals. Studies have shown that oxytocin is associated with social memory [111], affiliation [79, 142], and pair bonding in animals [31, 77, 78], and some researchers have suggested that oxytocin may also play a largely social role in human behavior [66].

Studies in humans are at an early stage but are gradually revealing some potentially useful results. In one of the

earlier studies, duration and pattern of social gaze (towards the eye region) in men were increased by administration of an intranasal dose of oxytocin [62]. Gaze is generally thought to be predictive of the ability to interpret the meaning of social situations and the intentions of others [84].

Research in social behavior has shown that a dose of intranasal oxytocin increases trust in social situations, suggesting that it might serve an affiliative purpose in humans, as well as animals [88] (especially among in-group members). For this reason, oxytocin is sometimes dubbed the "trust hormone". However, later work has shown that oxytocin modulates much more in social cognition than just interpersonal trust. fMRI studies have shown that oxytocin exerts influence on important neural circuits for a wide range of social–cognitive abilities such as eye gaze, mentalizing, emotion recognition, and learning [18, 46, 47, 57, 83, 89, 110, 117]. Regions in these studies which are affected by oxytocin, such as the amygdala, fusiform gyrus, ventromedial prefrontal cortex, insula, superior temporal gyrus, and inferior frontal gyrus [108], are consistently atypical in conditions where difficulties in social cognition are a defining feature as in ASC [45, 95]. Extensive reviews on oxytocin can be found elsewhere (e.g., [66, 128]).

Testosterone versus oxytocin administration

Due to the seemingly disparate effects of administering testosterone and oxytocin, it has recently been proposed that steroids and neuropeptides are important in different environments [27]. For example, testosterone may increase vigilance and motivation for action and may reduce social cognition in environments that demand action (such as in emergencies or high stress situations). Neuropeptides such as oxytocin may increase social cognition in environments that are safe or that do not demand action. The subtleties of these interactions are numerous and varied and need further testing [27]; however, it will be important to consider the environment and situational contexts when interpreting the findings from research of this kind, where the vast majority of studies are conducted in laboratory settings.

Further, unlike the testosterone studies that mainly include females, the vast majority of the studies on the effects of oxytocin have only included males. These samples were mainly chosen as a result of the practicalities of the side effects associated with each hormone. The generalizability of results from these studies has not yet been thoroughly tested, and possible sex-dependent outcomes have not been ruled out.

Future directions

In all the research described above, the role of the social environment has largely not been considered in depth. Social interactions undoubtedly play an important role in the development of social and emotional behavior. For example, research on gender-based expectations may cause parents, teachers, or caregivers to elicit and reinforce expected behavior from children [127], thus shaping the child's behavior. Further work on the role of the environment and how it interacts with hormone levels and behavior would be highly informative.

The relationships between hormones and behaviors in humans are likely to be dependent on many factors, and in the main report correlations with hormone levels measured at a single time point. Research in animals has generally shown that hormonal effects on behavior may be dose and time dependent [39, 71], and these issues need to be clarified. The replication of the results in larger sample sizes would also help to increase the range of hormone levels observed in these studies and assist in identifying any factors that are linked with levels in the extreme ranges.

It would also be valuable to further establish the relationships between direct measures of hormones (e.g., amniotic fluid or serum measures) and physical characteristics (e.g., 2D:4D ratio or dermatoglyphics) which have been used as proxy measures of hormone exposure. The benefit of using these types of measurements is that they are easy to obtain and have also been linked to multiple areas of human development. However, limited evidence exists for a relationship between these proxy measures and exposure to prenatal hormones. If such a link was confirmed using direct measures of hormones, it could simplify future investigations of hormone effects.

In studies of puberty, it would be beneficial for the field to include more in-depth studies that investigate the contribution of pubertal development, hormone levels, and social influences on development. The degree to which genetic variation is coupled with changes in hormone exposure is also unknown, and it may be that changes in hormone levels are simply a manifestation of a genetic influence. This would be an interesting area for future research since investigations of current testosterone levels have shown rates of heritability between 50and 66 % [65, 75]. Sex hormones also have an epigenetic role in changing gene expression throughout development and likely interact with sex chromosome effects on sexual differentiation [101, 102], and further exploration of applications to social behavior would be important.

With regards to the administration studies, it is worth noting that the majority of studies that have used this methodology have restricted their samples to either a female sample when using testosterone or a male sample when using oxytocin. As a result, the findings of the abovementioned administration studies may not necessarily be generalized to samples of the opposite sex. Future studies should compare the responses of males and females to ascertain whether there

may be any sex-dependent effects. The testosterone administration studies also include those who are using oral contraceptives, which itself is a hormone manipulation. It would be important for this area for studies to investigate how oral or hormone contraception may interact with the testosterone administration.

Conclusions

Research suggests that human social and emotional behaviors may be affected by gonadal hormones, in particular exposure to testosterone. The role of prenatal testosterone appears to be vital for early organization of the brain and, in particular, in the programming of sexual differentiation during critical periods of development. In humans, the most important period appears to be early–mid pregnancy. This finding has been repeated in studies looking at a number of behavioral measures and is also indicated by tendencies in those who are naturally exposed to elevated levels of the hormone through clinical conditions. It is also generally supported by studies in nonhuman mammals.

In later life, the effects of hormones such as testosterone during puberty have been shown to also predict behavior, but it is thought that these effects activate or fine-tune the early organization of the brain, although the exact relationships between these two time periods are far from clear. To some extent, the activational effect of hormones during puberty appears to be dependent on exposure during the organizational period of early development, when key tissues are first formed.

While the above conclusions appear to generally hold, it is apparent that there is still much work to be done to further understand the subtle effects that specific changes to hormone levels may have. Administration of hormones to an individual can provide some further clues. Generally speaking, such studies have concluded that increased testosterone levels seem to be involved with decreased social and emotional behavior, whereas administration of oxytocin increases social and emotional behavior.

More recent studies are beginning to identify the physical processes which may be involved in the effects of hormones on development and behavior. This research is generally at an early stage, though there is an indication that specific areas of the brain are more developed in those with higher prenatal testosterone levels. Functional MRI studies involving administration of particular hormones also indicate greater or reduced response from individual areas of the brain due to changes in testosterone or oxytocin levels. Such experiments are useful because they do not require a longitudinal design but at the same time cannot easily examine organizational effects. The ways in which steroids interact with neuropeptides and other hormones, as well as the cause of natural variation of sex steroids in general, is still not well understood.

The investigation of both organizational and activational hormone exposure on behavioral development remains an area needing much more detailed research. In addition to helping us map the process of human development, findings in this area could have major implications for clinical conditions characterized by social and emotional difficulties such as autism. Such conditions have a very major impact on an individual's quality of life.

Although it does not yet provide a clear way to help, the science examining hormonal effects on social and emotional development has come a long way and continues to evolve at a rapid pace. This remains a vibrant area of research where long-term commitment to research goals can greatly increase our understanding of human physical and behavioral development. Many exciting studies are underway, and we look forward to disentangling some of the many biological and environmental factors that contribute to shaping human social and emotional behavior.

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