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The early postnatal period, mini-puberty, provides a window on the role of testosterone in human neurobehavioural development

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Abstract

Experimental research in non-human mammals indicates that testosterone exposure during early periods of rapid brain development has enduring influences on brain and behaviour. These influences are exerted when testosterone is higher in developing males than females, and the affected characteristics are those that differ by sex. Testosterone is higher in males than in females from about weeks 8 to 24 of human gestation and then again during early infancy, and both of these periods are times of rapid brain development. Substantial evidence suggests that testosterone prenatally influences human neurobehavioral development. Emerging evidence suggests that the early postnatal period is important too. This early postnatal period could provide a window for studying testosterone interacting with experience to shape human gender development.

Highlights

- Testosterone during mini-puberty appears to influence later gender-typical play behaviour
- Testosterone during mini-puberty may also predict other gender-related behaviours
- Saliva samples can measure individual differences in testosterone at the peak of mini-puberty
- Saliva samples seem most informative when collected between weeks 4 and 12 postnatal
- Repeated sampling and the use of measures that show large gender differences could increase power

Introduction

Thousands of studies of non-human mammals have documented the important role of testicular hormones in sexual differentiation of the brain and of behaviour. These studies have shown that testosterone, and hormones produced from testosterone, exert enduring masculinizing and defeminizing effects during early sensitive periods of rapid brain development. The sensitive periods correspond to times when testosterone is higher in developing male than female animals, and, in many species, these periods occur prenatally as well as during early postnatal life. The characteristics that are affected by testosterone during these early sensitive periods include a range of behaviours that differ on average for male and female animals, as well as aspects of brain structure that differ by sex. (See [1;2] for reviews). This review outlines what we know about similar influences of testosterone on human development. It concludes that: 1. Testosterone, prenatally, influences human gender development; 2. Recent evidence suggests that testosterone during the early postnatal period of mini-puberty also influences human gender development; 3. This early postnatal period could provide an accessible window for studying the influence of testosterone in interaction with experience on human neurobehavioral development.

Testosterone during early human development

In humans, testosterone is higher in male than in female foetuses prenatally, particularly from about week 8 to 24 of gestation, and this difference appears to be maximal from about week 8 to 16 of gestation [3]. Testosterone is again higher in boys than in girls from about week 4 to 24 postnatal, and this difference appears to be maximal from about week 4 to 12 postnatal [4;5]. This early postnatal period of testosterone elevation is sometimes referred to as mini-puberty.

Human gendered behaviour

The behaviours that differ by sex in humans, and that thus might be influenced by testosterone during these early sensitive periods, can be referred to as gender-typical or gendered behaviours [2]. They include children's gender-typical play (toy and activity) preferences, as well as a person's direction of erotic interest (sexual orientation), and sense of self as male or female (gender identity) [6]. Although some other behaviours differ to some extent for men and women or girls and boys, these three (gender-typical childhood play behaviour, sexual orientation and gender identity) appear to show the largest differences [6]. To put them in a familiar context, the gender differences in play behaviour are similar in size to the sex difference in height, and the gender differences in sexual orientation and gender identity are many times larger than the sex difference in height. In contrast, gender differences in other behaviours, such as autistic traits, and expressive vocabulary in very young children, are typically less than half the size of the sex difference in height (Figure 1) [6].

Prenatal influences of testosterone on human behaviour

The available evidence suggests that testosterone prenatally influences human behaviours that show large gender differences. For instance, over a dozen studies from several independent research groups have found that girls exposed to unusually high concentrations of testosterone prenatally, because they have the genetic condition, congenital adrenal hyperplasia (CAH), show increased male-typical play behaviour and decreased female-typical play behaviour in childhood [7-14]. Several studies from independent research groups also suggest that females with CAH showed reduced heterosexual interest and reduced female-typical gender identity [8;10;14-16]. Studies of individuals with other genetic conditions that cause exposure to gender-atypical hormone concentrations prenatally are less numerous than studies of girls and women with CAH, but

they also suggest that testosterone prenatally influences later gender-typical play behaviour, sexual orientation and gender identity [2].

The strongest evidence supporting early influences of testosterone on human gender development has come from studies of individuals with genetic conditions, such as CAH, and these conditions have other consequences that also could influence behaviour. It is difficult, therefore, to be completely confident that early testosterone abnormality is the responsible agent. Evidence from a range of conditions, each with different consequences in addition to testosterone abnormality, provides some convergent evidence that testosterone is responsible for the behavioural changes [2]. Nevertheless, evidence that normal variability in testosterone exposure prenatally relates to later gendered behaviour would be useful too. Studies attempting to provide such evidence have looked at testosterone measured in amniotic fluid or maternal blood during gestation, or in umbilical cord blood at birth. Studies have even looked at the ratio of the 2^{nd} to the 4^{th} digit of the hands as a proxy for prenatal androgen exposure, because this ratio differs on average in males and females. All these approaches to measuring prenatal androgen exposure have limited reliability, however, and, perhaps unsurprisingly, they have produced largely inconsistent results [2;17;18].

Early postnatal testicular activation: mini-puberty

The early postnatal period when testosterone is elevated in developing boys (mini-puberty) might provide an alternative, and more accessible, window on the effects of testosterone on human gender development. It is easier to obtain samples from infants than from foetuses, facilitating direct sampling. In addition, reliability can be increased by repeated sampling. Recent evidence suggests that assessing testosterone during mini-

puberty could be a useful approach for studying the role of testosterone in the development of the brain and behaviour in typically-developing individuals.

Testosterone during mini-puberty influences the development of the male genitalia and reproductive function [19;20], and emerging evidence suggests that it contributes to later gender-typical play behaviour [5;21], and perhaps some aspects of verbal development [22;23], as well . Brain plasticity remains high throughout the early postnatal period [17]. Indeed, mini-puberty occurs during a period of particularly rapid brain development involving dramatic changes in total brain volume, grey matter volume, cortical thickness, cortical surface area, cortical lateralization, and cortical network development [24-27]. Thus, testosterone during mini-puberty could have substantial effects on the human brain and on human behaviour.

Mini-puberty and human gendered behaviour

Two studies of children's gender-typical play behaviour suggest links between testosterone during mini-puberty and later gendered behaviour. One study measured testosterone in urine samples at 1 week and 1 month postnatal, and then monthly until 6 months postnatal [5]. The area under the curve for testosterone (AUCt) during these first 6 months postnatal was higher in boys than in girls, and AUCt related significantly to later gender-typical play. Gender-typical play was assessed for 16 boys and 18 girls at age 14 months using a parent-report questionnaire on which higher scores represented more male-typical and less female-typical play. In addition, for 21 boys and 26 girls, time spent playing with individual gender-typical toys in a playroom was assessed. Among boys, testosterone related positively to male-typical scores on the parent questionnaire and negatively to observed play with a doll. Among girls, testosterone related positively to observed play with a truck.

A second study used physical development to estimate testosterone exposure prenatally, as well as postnatally [21]. This study measured ano-genital distance (AGD) and penile length at birth and at 3, 12 and 18 months postnatal in 81 typically-developing boys. AGD is the distance between the anus and the scrotum in boys, and between the anus and the vagina in girls. At birth, AGD is shorter in girls than in boys [5;28]. It also has been found to be influenced by exposure to testicular hormones prenatally [29;30]. Similarly, penile growth during the early postnatal period is influenced by testosterone during mini-puberty, and testosterone measured in serum at 3 months postnatal has been found to correlate with penile growth from birth to 3 months postnatal [19]. Thus, in this study, AGD at birth provided an estimate of prenatal testosterone exposure, and penile growth from birth to 3 months provided an estimate of early postnatal testosterone exposure (i.e., during mini-puberty). Both measures were significant, positive, and independent, predictors of male-typical play behaviour at aged 3 to 4 years, as assessed using the same parent-report questionnaire that was used in the study that measured testosterone in urine during mini-puberty. These results suggest that testosterone during mini-puberty contributes to later gender-typical play behaviour, at least in boys, and that the effects of testosterone during mini-puberty are independent of the well-established prenatal contribution of testosterone to gender-typical childhood play behaviour.

A second area of investigation has been language development. One study reported that testosterone measured at 4 weeks postnatal in blood samples from 18 boys and 18 girls showed the expected sex difference and appeared to relate to language lateralization at the same age. Testosterone measured at age 5 months in 11 boys and 9 girls from the same sample of children also was used to predict language performance at age 4 to 5 years, assessed using five subtests of a comprehensive battery. None of the five subtests assessing

language showed a gender difference in the 20 children, and only one of the five related to earlier testosterone concentrations [22]. These largely negative results could reflect the small sample and the use of language measures that did not show substantial gender differences. Another study from a different team found that testosterone measured in saliva samples from 36 boys and 42 girls at aged 1 to 3 months significantly predicted scores on a measure of expressive vocabulary at aged 18 to 30 months [23]. In this study, the measures of testosterone and of expressive vocabulary both showed significant sex / gender differences, and the significant correlation between testosterone and expressive vocabulary was seen in both the boys and the girls, separately.

Researchers have also examined the link between testosterone in infancy and later autistic traits, but these studies have produced largely negative results. Two studies related testosterone in saliva samples collected at 3 to 4 months of age to parent-reported autistic traits during toddlerhood. The first study found no correlation between testosterone and scores on the Quantitative Checklist for Autism in Toddlers (Q-CHAT) separately for 15 boys, or 20 girls, or for the entire sample of 35 children aged 18 to 35 months [31]. The second study found a significant positive correlation between testosterone and scores on an autistic traits subscale of the Brief Infant–Toddler Social and Emotional Assessment in a sample of 47 boys and 37 girls aged 18 to 24 months, but did not report any within-sex correlations [32]. Neither study found a sex difference in testosterone concentrations, perhaps because saliva samples were collected after the peak of mini-puberty. One of the studies also found no gender difference in autistic traits [32]. The lack of differences between boys and girls in testosterone in both studies and in autistic traits in one study limits the studies' implications for the neurobehavioural effects of mini-puberty on gendered behaviour, because testosterone is hypothesized to be influential at times when it is higher in males than in

females, and to influence characteristics that show gender differences. The third study measured testosterone at the peak of mini-puberty, between aged 1 to 3 months, but also found no relationship to subsequent Q-Chat scores in 39 boys, 47 girls or the entire sample of 86 children aged 18 to 30 months, although both salivary testosterone and scores on the Q-CHAT showed the expected sex / gender differences [33].

Methodological considerations

In studies exploring the impact of testosterone on human development, it is important both to measure testosterone in a reliable way, at a time when it differs in males and females, and to measure outcome variables that differ on the average for males and females. The bigger these gender differences in behaviour are, the more statistical power the study is likely to have. This relationship between power and the size of the gender difference may explain why the most consistent links between testosterone during mini-puberty and later behaviour have been seen for gender-typical play behaviour, because the measures used in these studies show large gender differences. A lack of power could also explain the inconsistent, but largely negative, results from studies relating testosterone during mini-puberty to later autistic traits. Measures of autistic traits show only small to moderate sex differences, and so large samples may be needed to detect relationships. Neither sexual orientation nor gender identity has yet been studied in relation to testosterone during mini-puberty. Given the large size of the gender differences in these characteristics, they would be promising candidates for investigation.

In regard to measuring testosterone during mini-puberty, the available evidence suggests that the difference in testosterone concentrations between male and female infants is largest from about 4 to 12 weeks postnatal, and that measuring testosterone after this time in saliva samples does not show a sex difference, at least in samples of similar size

to those studied so far. Urinary sampling, combined with tandem mass spectrometry for testosterone, appears to produce a larger sex difference, with significant differences between boys and girls as late as 5 months postnatal [5]. Similarly, blood samples appear to show sex differences in testosterone in infants as old as 5 months postnatal [22]. Measurement of the physical parameters of AGD at birth and penile growth from birth to 3 months postnatal also has produced promising findings, and this approach has the advantage of allowing investigation of the independent effects of testosterone prenatally and postnatally. Future research can investigate the advantages and disadvantages of each of these approaches to understanding the role of testosterone during mini-puberty in human gender development.

Conclusions

Current perspectives on human gender development emphasize that several different types of contributory factors, including not only early testosterone exposure, but also social and other types of experience, interact over time to produced gendered behavioural outcomes [2]. Research investigating these interactions has been challenging, in part because individuals who have experienced atypical testosterone exposure early in life, such as girls or women with CAH, are not numerous. A reliable approach to measuring testosterone exposure during early development in typically-developing individuals would enable research on interactive effects by facilitating assessments in large samples. For instance, measurement of testosterone during mini-puberty, using blood or urine, or saliva at the peak of mini-puberty, could enable studies investigating how early testosterone exposure interacts with other factors involved in gender development during early life.

Figure Captions:

Figure 1. Some, but not all, gender differences in human behaviour are large. The familiar sex difference in height is about 2.0 standard deviations (d) in magnitude. Some behavioural gender differences are as large, or larger, than the sex difference in height. Characteristics that show large differences include gender identity, sexual orientation, and children's gender-typical play styles, and toy interests (e.g., interest in dolls vs toy vehicles). In contrast, some behaviours that have been studied in relation to early postnatal testosterone exposure, such as autistic traits and expressive vocabulary in very young children, show substantially smaller gender differences (0.4 d) than that seen in height. (In the behavioural sciences, group differences of 0.8 d or greater are considered to be large, those of 0.5 d are considered to be of medium size, and those of 0.2 d are considered to be small [34].)

Summary figure. The early postnatal period of testicular activation may provide an accessible window for studying the role of early testosterone exposure in human gender development. There are two periods during early human development when testosterone is higher in developing males than females. These periods occur from about week 8 to 24 of gestation and from about the first to the sixth month postnatal. Both of these periods occur during times of rapid brain development. It is well-established that testosterone during the first of these periods influences later gendered behaviour, including gender-typical play, sexual orientation, and gender identity. There is recent evidence suggesting that testosterone during mini-puberty, just after birth, also influences gendered behaviour, particularly gender-typical play. This early postnatal period when testosterone is elevated is more accessible than the prenatal period, and so could provide a valuable opportunity for

studying the role of testosterone in human neurobehavioural development.

Competing Interests

The authors declare that they have no competing interests.

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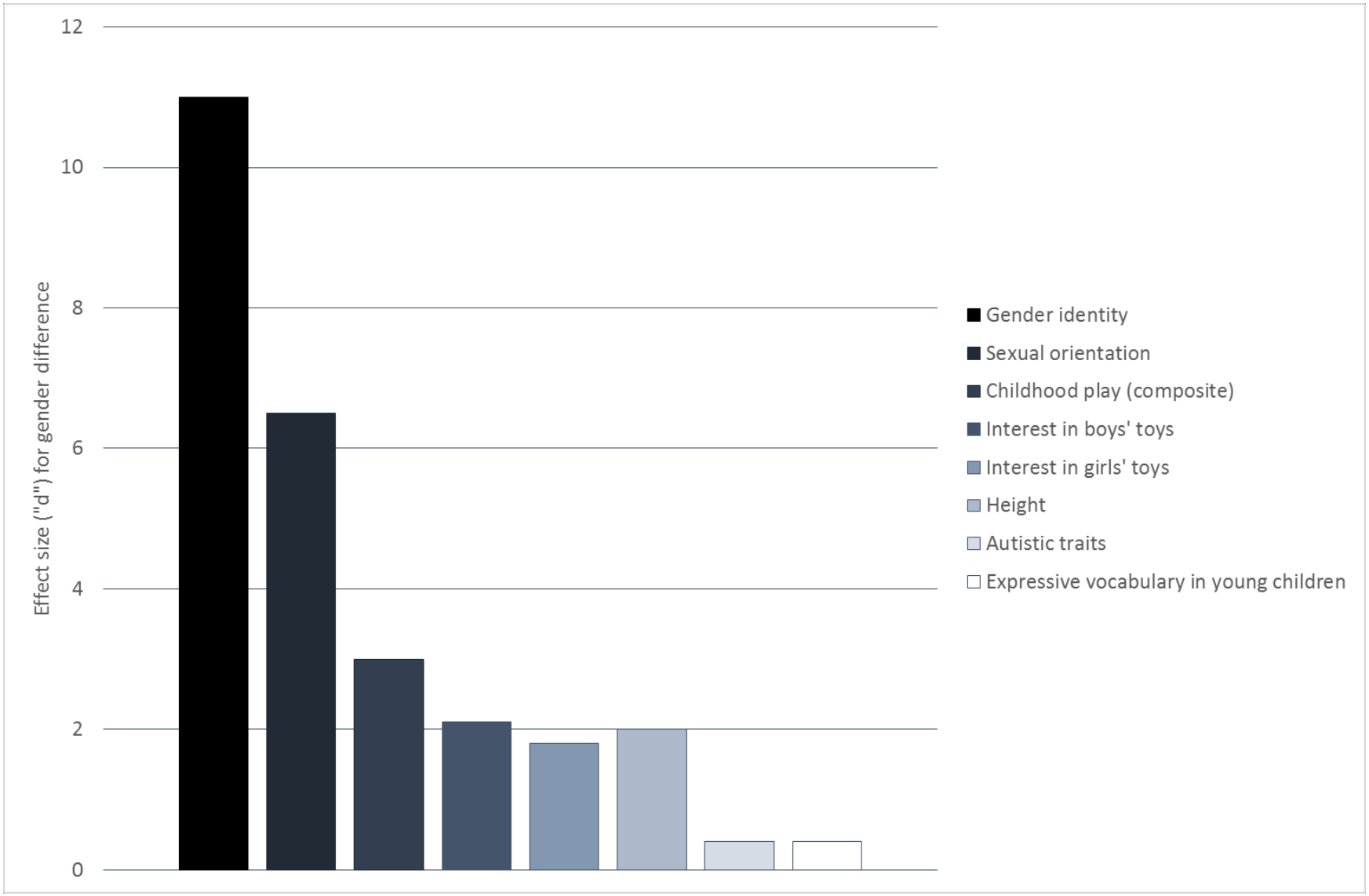
Figure 1


12
10
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Effect size ("d") for gender difference
Gender identity
Sexual orientation
Childhood play (composite)
Interest in boys' toys
Interest in girls' toys
Height
Autistic traits
Expressive vocabulary in young children

Summary figure

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