Review

Prenatal endocrine influences on sexual orientation and on sexually differentiated childhood behavior

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ABSTRACT

Both sexual orientation and sex-typical childhood behaviors, such as toy, playmate and activity preferences, show substantial sex differences, as well as substantial variability within each sex. In other species, behaviors that show sex differences are typically influenced by exposure to gonadal steroids, particularly testosterone and its metabolites, during early development (prenatally or neonatally). This article reviews the evidence regarding prenatal influences of gonadal steroids on human sexual orientation, as well as sex-typed childhood behaviors that predict subsequent sexual orientation. The evidence supports a role for prenatal testosterone exposure in the development of sex-typed interests in childhood, as well as in sexual orientation in later life, at least for some individuals. It appears, however, that other factors, in addition to hormones, play an important role in determining sexual orientation. These factors have not been well-characterized, but possibilities include direct genetic effects, and effects of maternal factors during pregnancy. Although a role for hormones during early development has been established, it also appears that there may be multiple pathways to a given sexual orientation outcome and some of these pathways may not involve hormones.

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1. Introduction

Sexual orientation varies markedly for men versus women, as well as for individuals within each sex. The great majority of women are androphilic (erotically interested in males), whereas the great majority of men are not. There also is variability within each sex. Some women are not androphilic, whereas some men are. The effect size (in standard deviation units or "d" [23]) for the sex difference is very large (d = 6.0–6.7) [66,67,103]. To put this magnitude in a familiar context, it is several times as large as the sex difference in height (d = 2.0) [69].

In regard to within sex variability, Kinsey suggested that about 10% of men and 5% of women are bisexual or homosexual. More recent research suggests that 2–6% of men in the United States, France and Great Britain have had homosexual experience [14,83,131]. A 1995 study examined homosexual orientation in terms of attractions as well as behavior, and in men as well as in women, and reported that 16–21% of men and 17–19% of women in the same three countries had experienced sexual attraction to individuals of their own sex [125]. Rates for actual sexual behavior in the past 5 years were also higher than in the other recent studies. They were 6.2% for men and 3.6% for women in the United States, 10.7% for men and 3.3% for women in France, and 4.5% for men and 2.1% for women in the United Kingdom. Regardless of the precise numbers, it is clear that there is a good deal of within sex variability in sexual orientation, and that a substantial minority of both sexes have some erotic interest in individuals of their own sex.

The origins of variability in sexual orientation are far from completely understood. The existence of the dramatic sex difference suggests that gonadal hormones, particularly testosterone, might play a role, given that testosterone plays an important role in the development of most, perhaps all, behavioral sex differences in other species. This article will review the evidence that testosterone, or other gonadal hormones, influence human sexual orientation. It will begin by summarizing information from experimental research in other species, because this research underpins the hypothesis that gonadal hormones might influence human sexual orientation. It will then critically evaluate the studies that have attempted to elucidate the role of the early endocrine environment in human sexual orientation. Finally, some directions for future research will be discussed.

Other articles in this special issue will focus in depth on some of the areas covered more briefly here. For instance, several authors will address the molecular and neural mechanisms involved in sexual differentiation of behavior as identified from basic research in a range of non-human mammals (papers by McCarthy, by Flanagan-Cato, by Tobet, and by Wallen). In addition, other authors cover sex differences in human brain structure and brain responses (papers

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by Swaab and by Savic-Berglund), the possibility of direct genetic influences on sexual orientation (paper by Bockland and Vilain) and evidence linking birth order, and perhaps maternal factors during pregnancy, to sexual orientation in men (paper by Bogaert). These treatments will not be duplicated here. Instead, the focus will be on the compelling evidence for a role of gonadal steroids in the development of human sexual orientation and associated childhood sex-typed behaviors.

2. Hormones and sexual differentiation of brain and behavior in non-human mammals: The empirical basis for hypothesizing that gonadal hormones influence human sexual orientation

As detailed in other articles in this issue, the early (prenatal, neonatal) hormone environment has powerful influences on neural and behavioral sexual differentiation in a wide range of mammalian species. Literally thousands of experiments involving manipulations of hormones have shown these effects, and the evidence will not be reviewed in detail here. However, this section will provide a summary of some of the general conclusions that are most relevant to a discussion of possible early endocrine influences on human sexual orientation.

First, genetic information determines whether the primordial gonads, which are originally identical in males and females, develop as testes or ovaries. This is called sex determination. Once the gonads have developed as testes or ovaries, their hormonal products, particularly testicular hormones, determine physical development as male or female, a process called sexual differentiation. In human beings, the testes become active at about week 7–8 of gestation [119,129]. Testicular hormones cause the external genitalia to develop in the male pattern (penis and scrotum), and in the absence of testicular hormones, the external genitalia develop in the female pattern (clitoris and labia) [149]. As outlined below, similar processes of sexual differentiation under the control of gonadal steroids occur in the mammalian brain during early development.

Gonadal steroids have two general types of influences on brain and behavior, and they have been called organizational effects or influences and activational effects or influences [116]. Organizational and activational effects are distinguished by their timing and their permanence. Organizational influences typically occur early in life, particularly prenatally and neonatally, and they are enduring. In contrast, activational influences occur later in life, typically in adulthood, and they are transient, waxing and waning as hormone levels rise or fall. An example is the activation of female sexual behavior by estrogen and progesterone near the time of ovulation, and the decline in sexual behavior at other phases of the cycle, when these hormones decline. This article focuses largely on the early and enduring, organizational influences of hormones on human sexual orientation and childhood sex-typed behavior.

Several general principles of organizational influences are worth noting. One is that they typically occur during critical, or sensitive, periods of development, and these periods occur at times when testosterone is elevated in developing male animals. One implication of this general principle is that the hormone must be present at the appropriate time to have its effect. If it is present too early or too late, the impact will not be the same. In addition, although present only briefly, the impact of exposure to the hormone at the appropriate time can persist across the lifespan. These early, time-linked, persistent effects are thought to occur because hormones direct some aspects of neural development during early life [3], influencing cell survival, neuroanatomical connectivity and neurochemical specification [97]. Another general principle is that the times when testicular hormones influence behavioral sexual differentiation in a given species correspond to the times when testosterone is elevated in males compared to females of that species.

It is hard to get information on hormone levels in developing humans. However, the small amount of available information examining testosterone levels in human fetuses and neonates suggests that there is a marked elevation in testosterone in males compared to females beginning by about week 8 of gestation and tapering off by week 24, and again shortly after birth, from about the first to the third month of infancy [118,129]. Testosterone measured in amniotic fluid samples also is higher in male than in female fetuses, particularly near midgestation, with one study finding a significant sex difference in samples taken between gestational weeks 15 and 21, but not weeks 36 and 40 [20]. Similarly, another study found that amniotic fluid testosterone was highest in males between about weeks 12–18 of gestation, and was significantly higher at this time than later in gestation [142]. This study also found that testosterone continued to be higher in males than in females even late in gestation, however, although the sex difference attenuated at later gestational ages. There is some dispute about the timing of the early postnatal sex difference in testosterone. Although an early study, using blood samples, suggested a peak from 1 to 3 months postnatal [129], a subsequent study, using saliva samples, instead found that testosterone was highest just after birth and declined to baseline by about 3–4 months postnatal [76]. Regardless of the timing of this sex difference, testosterone appears to be higher very early in infancy in boys than in girls. The gonads are relatively quiescent following this early testosterone elevation in boys, until of course, the dramatic sex differences in testosterone and other gonadal steroids that begin at puberty [43].

According to the classical model of hormonal influences on mammalian sexual differentiation, prenatal or neonatal exposure to testicular hormones causes male-typical development, whereas female-typical development occurs in the absence of testicular hormones. A corollary of this formulation is that ovarian steroids are not required for female-typical development, a point to which we will return. The empirical record generally supports this model for a wide range of brain regions and behaviors that differ on the average for male and female animals. For example, treating female rodents with testosterone early in life decreases their female-typical behavior in adulthood, and increases their male-typical behavior. Similarly, castrating male rodents early in life leads to decreased male-typical, and increased female-typical, behavior subsequently [54]. The same manipulations produce neural changes as well. Early testosterone treatment of female rats increases the volume of sexually-dimorphic nucleus of the preoptic area (SDN-POA), a region of the anterior hypothalamic preoptic area that is larger in male than in female rats [50,51], and castration of male rats early in life reduces the volume of the same nucleus [50,80].

In contrast to the effects on male animals of removing the testes early in life, removal of the ovaries at this time does not have substantial effects on sexual differentiation of female animals. It does not, for instance, reduce their ability to show female-typical sexual behavior in adulthood, assuming appropriate activating hormones are replaced at that time, nor does it increase (i.e., masculinize) the volume of the SDN-POA [80,89]. In addition, many of the influences of early exposure to testosterone on sexual differentiation of brain and behavior in rodents occur after the testosterone has been converted within the brain to estrogen. This conversion occurs via the action of the enzyme, aromatase. Neural and behavioral masculinization by estrogen is surprising and counter intuitive, and it is an excellent example of scientific research revealing something that would otherwise not have been expected. The evidence is convincing, however, at least in some species. Treating developing female rats with estradiol, or other estrogens, does not have feminizing effects, but the opposite. The effects of estrogens resemble those of testosterone, increasing male-typical sexual behavior and the volume of the SDN-POA, and reducing female-typical sexual behavior

[31,54,93]. Additional evidence that estrogen plays a role in neuro-behavioral masculinisation early in life comes from evidence that blocking conversion of androgen to estrogen, or blocking estrogen receptors, has some of the same effects as castration, even in the presence of high levels of testosterone [22,33,96,98].

Another relevant point about organizational influences of hormones is that they can vary in their specific mechanisms for different sexually differentiated behaviors. For example, in the rat, the critical periods for hormonal influences on male-typical and female-typical sexual behavior differ somewhat [21]. Consequently, by timing hormonal manipulations to hit or avoid periods during which each behavior differentiates, animals can develop to show female-typical but not male-typical behavior (conventionally feminine animals), male-typical but not female-typical behavior (conventionally masculine animals), male-typical and female-typical behavior (bi-potential animals), or neither male-typical nor female-typical behavior (asexual animals).

The effects of hormones are not limited to behaviors that are closely linked to reproduction, although the majority of studies have focused on reproductive behaviors. When other behaviors that show sex differences, meaning that they differ on the average for male and female animals, have been studied, however, they also have generally been found to be influenced by early manipulations of testicular hormones. Behaviors that have been found to be sensitive to the early hormone environment include rough-and-tumble play in juvenile rodents and non-human primates, and aggression, parenting behaviors and maze learning in rodents [8,54,69].

The specific mechanisms of sexual differentiation vary for different behavioral endpoints. As mentioned above, the timing of maximal sensitivity varies for male-typical and female-typical reproductive behaviors. Also, each of the other behaviors that are influenced by early hormone exposure are likely to have somewhat different periods of maximal sensitivity as well, depending on when relevant neural systems are undergoing hormone-dependent developmental processes. In addition, different behavioral endpoints appear to be sensitive to different metabolites of testosterone. Although masculine and feminine reproductive behaviors, for instance, are influenced in the rat by estrogen derived from testosterone, masculine-typical rough and tumble play appears to be influenced by testosterone itself, or by dihydrotestosterone produced from it via the enzyme, 5 alpha reductase [100]. Processes that occur after the hormone interacts with the receptor also can differ for different brain regions and behavioral outcomes. For instance, in rats, estradiol interacts with estrogen receptors to enhance male-typical sexual behavior and dendritic spine formation in the preoptic area by influencing synthesis of prostaglandin E2 (PGE_2), whereas PGE_2 does not appear to be involved in a second neural region, the ventromedial nucleus of the hypothalamus, where estradiol also acts through estrogen receptors to enhance male-typical neural structure [97].

Although male and female sexual behaviors have been studied more thoroughly than other sex-related behaviors, it is likely that each aspect of behavior that is influenced by the early hormone environment is regulated by somewhat different neural circuitry and involves somewhat different specific mechanisms. These different specific mechanisms could include, for example, different times of maximal sensitivity, sensitivity to different metabolites of testosterone and involvement of different downstream processes, such as the involvement of PGE_2. Overall, nevertheless, studies where hormones have been manipulated experimentally in a wide variety of mammalian species, ranging from rodents to rhesus monkeys, indicate that exposure to high levels of testosterone during critical periods of early development promotes male-typical neural and behavioral development, whereas exposure to lower levels of testosterone leads to female-typical neural and behavioral development. Given the large sex difference in human sexual orientation, the hypothesis that the early hormone environment influences this sexually differentiated outcome merits investigation.

3. Definition and assessment of sexual orientation in humans

Sexual orientation typically is conceptualized as the direction of erotic interest – in males (androphilic), in females (gynephilic), or in both. In addition to these three categories, some individuals do not report erotic interest in either sex. Even these three (or four!) seemingly simple categorizations can become complicated, for instance, by including quantitative and qualitative assessments, by making assessments over the most recent 12 months or the lifetime, by assessing behavior or fantasy or identity, or by conceptualization as homosexual or heterosexual, rather than androphilic or gynephilic.

Very simple assessments might ask an adult if they live with a partner, and what sex that partner is. Another simple assessment might ask a person whether he or she identifies as heterosexual, homosexual or bisexual. Generally, however, it is thought preferable to assess more than one dimension of sexuality, asking about sexual interest in fantasy, as well as in behavior. The rationale here is that a person might have homosexual or bisexual interests, but not have the opportunity to express these behaviorally. Similarly, occasional homosexual fantasy or behavior might not lead to self identification as non-heterosexual. Therefore, it is generally agreed that fantasy or imagery should be assessed rather than only actual behavior or self-described identity, as homosexual, heterosexual or bisexual, alone. As will be seen, however, this has not always been done.

Kinsey scales [85,86] are a common assessment tool for quantifying sexual orientation in behavior and fantasy or imagery. These scales can be applied to interview data to create the following groupings: 0 = entirely heterosexual; 1 = largely heterosexual but incidentally homosexual; 2 = largely heterosexual but also distinctly homosexual; 3 = equally heterosexual and homosexual; 4 = largely homosexual but also distinctly heterosexual; 5 = largely homosexual but incidentally heterosexual; and 6 = entirely homosexual. Because the distribution of Kinsey scale scores is highly asymmetric, they are often dichotomized for statistical comparisons, with those scoring 0–1 (exclusively and almost exclusively heterosexual) forming one group and those scoring 2–6 forming the other.

It also is important to distinguish sexual orientation from other aspects of sexual identity, including core gender identity, and gender role behaviors. Core gender identity is the sense of self, usually as male or female, but occasionally as intersex or neither. Gender role behaviors are those behaviors that are culturally associated with one sex or the other. They also can be defined empirically as behaviors that show sex differences, meaning that they differ on the average for males and females in a given cultural group. These three categories of behavior might be expected to be consistent, but this is not always the case. Although many men understand themselves to be male, are interested in female sexual partners, and engage in male-typical behaviors in other areas, such as watching or playing football, this is not always so. The situation is similar for women, as well as for girls and boys. Behavior across these three categories, or across the many types of behavior included in the concept of gender role behavior, is not always consistent for a given individual. Nevertheless, there is some consistency, and, as will be discussed later in this chapter, the hormonal factors that influence sexual orientation also influence other aspects of sex-typed behavior, including childhood toy, playmate and activity preferences.

Another complication arises because the terms homosexual and heterosexual are informative only if one knows the sex of the person in question. This becomes apparent, for example, in discussing transgendered individuals (individuals who feel that their apparent sex contradicts their core gender identity). If a male to female trans person is attracted to females, is she homosexual or heterosexual? Thus, the terms androphilic (erotically interested in males) and gynephilic (erotically interested in females) can be useful.

4. Do hormones have activational influences on sexual orientation in adulthood?

There are no apparent hormonal differences in adulthood between men who are heterosexual and those who are not [101]. Similarly, no such differences have been reported consistently in heterosexual compared to non-heterosexual women, although there may be a slight elevation of androgens in some homosexual women [16,102]. Consistent with these findings, men who become hypogonadal in adulthood do not change their sexual orientation, though their sexual interest declines [101]. Similarly, although men and women sometimes are treated with androgen or estrogen for medical reasons, this does not appear to alter their sexual orientation.

5. Sources of information on prenatal hormones and sexual differentiation of human behavior

Although the adult hormone environment does not seem to influence sexual orientation, this does not mean that the early hormone environment is also without effect. Studying possible influences of the prenatal or neonatal hormone environment on human behavior is challenging. It would be unethical to administer hormones experimentally to pregnant women to test for influences on sexual orientation. However, some disorders of sex development (DSD) [75] involve alterations in the hormone environment, beginning prenatally. In addition, women have sometimes been prescribed hormones during pregnancy. Both of these types of situations provide information on the consequences of dramatic alterations in hormones prenatally for human behavioral development, including the development of sexual orientation. Information also has come from studies where hormones have been measured during early development, for example in the amniotic fluid or in the maternal blood, and related to later behavior. Some studies also have related physical characteristics that are thought to relate to prenatal androgen exposure, particularly finger ratios, to behavioral outcomes, using these physical characteristics as bio-assays of prenatal androgen exposure. Finally, some evidence has come from studies of the impact of stress, which causes changes in adrenal hormones, including androgens, during gestation on sexual orientation of offspring. Each of these types of influences will be described in more detail in subsequent sections.

5.1. Congenital adrenal hyperplasia (CAH) and sexual orientation

The most extensively studied DSD in regard to sexual differentiation of behavior is congenital adrenal hyperplasia (CAH). CAH is an autosomal, recessive disorder that occurs in approximately 1 in 5000 to 1 in 15,000 births in Europe and North America [110]. In about 95% of cases, the disorder results from mutations in the CYP21A2 gene that encodes the enzyme, 21-hydroxylase (21-OH). The deficiency in 21-OH impedes cortisol production, and results in shunting of cortisol precursors into the androgen pathway and therefore overproduction of adrenal androgens. Androgen levels in female fetuses with classical CAH, the form of the disorder known to involve prenatal androgen elevation, are raised

dramatically [114,152], and, as a consequence, girls with classical CAH are born with some degree of genital virilization. In rare cases, the virilization is sufficiently severe that they are mistaken for boys at birth [108]. Usually, however, the genital ambiguity leads to prompt diagnosis and postnatal treatment to regulate hormone levels, and assignment and rearing as girls, with surgical genital feminization, typically in infancy.

At least 10 studies have been published in the English language on sexual orientation in girls and women with CAH in comparison to female controls [29,44,45,67,82,88,95,103,109,155]. The overwhelming conclusion from these studies is that women with CAH are less likely to be exclusively or almost exclusively heterosexual than are other women. The studies have used varied methodologies to assess sexual orientation and have looked at different age ranges, and these differences appear to influence the results obtained. Reduced heterosexual orientation is more likely to be observed in studies assessing erotic imagery, as well as, or rather than solely, the sex of actual sexual partners [103]. In addition, reduced heterosexual orientation is more likely to be found in studies that are restricted to individuals 16 years of age or older, than in studies that include children. For instance, in one study that reported data for various age groups [29], 20% of females with CAH in the age range 11–41 years indicated that they had "had or wished to have a long term/ steady relationship with a female partner", whereas the comparable figure for those over 16 years of age was 26%, and for those over 21, it was 44%. This age related difference may occur because individuals become more aware of, and comfortable with, their sexual orientation as they develop from early adolescence into adulthood.

Severity of the CAH disorder also relates to outcomes for sexual orientation. This has been demonstrated in a study of 143 women with CAH, ages 18–61 years, compared to 24 control women who were unaffected sisters or female cousins of women with CAH [103]. Sexual orientation was assessed using interviews regarding both erotic imagery and behavior. Looking at data for lifetime, overall sexual responsiveness, which combines imagery and behavior, among 38 women with the most severe, salt losing form of CAH, 41% were not exclusively or almost exclusively heterosexual, whereas the comparable figure for 21 women with the less severe, simple virilizing CAH was 29% and for unaffected controls it was 5%. Figures for actual sexual experience with a partner of the same sex were lower, but showed the same pattern, with 21% of women with salt losing CAH having had experience with same sex partners, compared to 5% of simple virilizers and 0% of unaffected women. Surprisingly, 79 women with non-classical CAH, which is thought not to cause elevated androgen prenatally, showed altered lifetime, overall sexual responsiveness, with 24% not exclusively or almost exclusively heterosexual, and 4% reporting sexual experience with a partner of the same sex.

Another recent study of 62 women with CAH, ages 18–63 years, compared to 62 age-matched controls, also found a relationship between disease severity and sexual orientation in women with CAH. This study assessed sexual orientation in terms of response to a single item on a paper and pencil questionnaire scored as "homosexual", "bisexual" or "heterosexual" [44], and related this to CAH severity as indicated by genotype. Among women with the most severe, null mutation of the CYP21A2 gene, 50% indicated that they were bisexual or homosexual, whereas for those with the next most severe, 12 splice mutation, the comparable figure was 30%. These two mutations correspond roughly to the salt losing variant of CAH, and for the two mutations together, 35% of the women reported being either bisexual or homosexual. For women with the I172N mutation, which is associated with the simple virilizing form of CAH, 5% reported that they were not heterosexual, and for the least severe V281L mutation, associated with non-classical CAH, the same figure was 0%. This last group was very small,

including only five women, but the result contrasts with the prior unexpected finding [103] of decreased heterosexual orientation in women with non-classical CAH.

Only one of the 10 studies [88] did not find elevated rates of non-heterosexuality in women with CAH. This study included 45 patients, many with milder forms of CAH. Only 45% had salt wasting CAH, and 17% had the non-classical form of the disorder. In addition, the assessment of sexual orientation might not have been sufficiently sensitive. The assessment procedure was not described in detail, but it appeared to involve response during an interview to a question about sexual orientation identity and perhaps a question about the sex of any co-habiting partner. In addition, women might not have felt sufficiently comfortable to reveal their sexual orientation. In regard to outcomes for sexual orientation, the authors say "Two patients and 1 control individual stated they were lesbians and lived with a female partner. One of the women stated in the questionnaires that she was a lesbian but denied it in the personal interview. Whether this was a sign of shyness or instability in her decision remains unclear." [88].

In addition to being associated with reduced heterosexual orientation, CAH is associated with reduced sexual activity and interest in general [44,155]. This may occur in part as a consequence of the genital virilization at birth and poor outcomes of surgeries to feminize the genitalia [26,27,107]. For instance, in explaining their reduced sexual activity, individual women with CAH have been reported to indicate "nobody wants someone like me", or that they "have not dared to take that step" [44]. In regard to reduced interest, individuals have cited "looking different", or "inability to relax when partner is not told (about the disease)" [44]. A negative impact of CAH on a person's sex life has also been attributed to "pain and bleeding during intercourse" [44]. Based on consideration of these types of physical problems, it has been suggested that decreased heterosexual interest and activity in women with CAH might result partly from the physical consequences of CAH.

6. Prenatal exposure to estrogen and sexual orientation in women

As noted above in the section on animal models of hormonal influences, exposure of rodents to estrogenic hormones during early development has many of the same neural and behavioral influences as exposure to androgenic hormones, but does not typically cause genital virlization [31,53,71,73]. Therefore, studies of women exposed to high levels of estrogens prenatally could provide information regarding effects of early exposure to masculinizing hormones on sexual differentiation of brain and behavior independent of influences on the external genitalia. Diethylstilbestrol (DES) is a synthetic estrogen that was widely prescribed to pregnant women in the 1940s, 1950s and 1960s, in the mistaken belief that it would help to prevent miscarriage, and it is thought that DES was prescribed to 1–5 million pregnant women in the United States during this period [62,111]. It was also used in other countries, and offspring from DES-treated pregnancies have provided information of the effects of prenatal exposure to estrogen on sexual orientation, in the absence of genital virilization or genital surgery.

One research team has studied three samples of women exposed prenatally to DES. The first sample included 30 women exposed to DES, 30 unexposed women recruited from the same gynaecological clinic and 12 unexposed sisters of the DES-exposed women [39]. All of the participants had abnormal PAP smear findings. (Although DES rarely, if ever, causes genital virilization, prenatal exposure is often associated with abnormal PAP smears [63].) Sexual orientation was assessed by interview and rated using Kinsey scale scores, and a global rating for lifelong sexual

responsiveness (behavior and fantasy combined) was reported for 29 of the DES-exposed women and 30 of the controls. DES exposure was associated with reduced heterosexual orientation. Although 76% of the DES-exposed women were exclusively or almost exclusively heterosexual for lifetime scores, 24% were not. The comparable figure for the matched controls with abnormal PAP smear findings was 0%. The subset of 12 sister pairs showed a similar pattern with 42% of the DES-exposed sisters being not exclusively or almost exclusively heterosexual for their lifetime in terms of fantasy or behavior, compared to 8% of their unexposed sisters. Among the total group of DES-exposed women, five had experienced homosexual activities involving genital contact and two were living with a female partner. The same research team later reported data from this initial study along with data from two more samples of women exposed to DES prenatally [105]. The first additional sample included 30 DES-exposed women, 30 demographically matched controls, with no history of DES-exposure or abnormal PAP smears, and 8 unexposed sisters. In this sample, a global Kinsey rating for lifelong sexual responsiveness was reported for 29 of the DES-exposed women and 30 of the matched controls. For the exposed group, 35% were not exclusively or almost exclusively heterosexual, whereas for the control group the comparable figure was 13%. Among the 20 sister pairs in the first and second samples, 40% of the DES-exposed group, compared to 5% of their sisters, were not exclusively or almost exclusively heterosexual. The second additional sample included 37 DES-exposed women whose mothers' obstetrical files indicated prescription of at least 1000 mg of DES during the pregnancy, and age-matched daughters of women from the same obstetrical practice, whose mothers' files showed that no DES was prescribed. For these women, 16% of the DES-exposed group and 5% of the unexposed group were not exclusively or almost exclusively heterosexual. For all three samples combined, 24% of the DES-exposed women, and 6% of the control women were not exclusively or almost exclusively heterosexual.

A separate investigation of women exposed to DES prenatally concluded that this exposure did not influence their sexual orientation. This study included 3946 women exposed prenatally to DES and 1740 women not exposed to DES [134]. The DES-exposed women were somewhat less likely than the unexposed women to have had sex with a female partner. The DES-exposed women also were more likely than the unexposed women to have ever married, and for those who had had sexual intercourse with a man, were less likely to have had sexual intercourse before age 17 or to have had more than one sexual partner. These last differences raise questions about the comparability of the exposed and unexposed groups, and, although the large sample is impressive, the assessment of sexual orientation, in terms of a single question regarding sexual behavior, is relatively insensitive.

7. Prenatal exposure to ovarian hormones and sexual orientation in men

The possibility that exposure to ovarian hormones before birth influences sexual orientation in males also has been investigated. These studies have produced largely negative results. One study compared two groups of men exposed to the synthetic estrogen, DES, prenatally to matched controls [84]. One group included 17 men exposed to DES alone and the second included 21 men exposed to DES along with natural progesterone. The study also included 10 men exposed prenatally to natural progesterone alone and 13 men exposed prenatally to synthetic progestins alone. Each of these groups was compared to matched controls. None of the four groups of hormone-exposed men differed from their respective controls in sexual orientation in fantasy or behavior.

In addition, for all four samples combined, non-heterosexual orientation was reported by 8 of the 61 hormone-exposed men (13%), and by 8 of the 60 control men (13%).

Two other research teams also have looked at sexual orientation in men exposed to DES prenatally, and have found no evidence of reduced heterosexual orientation. One [106] studied 46 men exposed to DES and 29 unexposed controls. Men exposed to DES had somewhat more heterosexual coital experience than did controls, but did not differ in the number of heterosexual or homosexual coital partners. The second [134] compared 1342 DES-exposed men to 1342 unexposed men, and found no difference in the numbers reporting sexual experience with a partner of the same sex, although, as noted above, this study used a relatively insensitive procedure for assessing sexual orientation. Nevertheless, the findings overall suggest that prenatal exposure to estrogen does not feminize sexual orientation in developing males, and this conclusion is consistent with predictions from results of experimental studies in other species, where early exposure of male animals to estrogen does not promote the development of female-typical behavior.

7.1. Androgen deficiency and sexual orientation in men

Some research also has investigated the influence of early androgen deficiency on sexual orientation in men. We have less information about such effects in men than about the effects of elevated androgens in women, however, perhaps because disorders causing reduced androgen exposure in males are even rarer than disorders causing increased androgen exposure in females. Relevant information has come from several DSD, however, including androgen insensitivity syndrome (AIS), and syndromes causing enzymatic deficiencies in the androgen pathway.

AIS involves deficiency in the ability of androgen receptors to respond to androgens [58]. It is transmitted as an X-linked genetic trait and so occurs almost exclusively in XY individuals. In the complete form of AIS (CAIS), the testes produce normal amounts of androgens, but the affected individual appears female at birth, because the external genital structures have been unable to respond to the androgens. These XY individuals are typically assigned and reared as girls with no suspicion of the underlying disorder or the Y chromosome. At puberty, estrogen derived from testicular androgens causes female-typical breast development. The disorder is usually diagnosed after menstruation fails to occur, because of the lack of female internal reproductive structures. Sexual orientation in XY women with CAIS appears not to differ from that of women in general. Women with CAIS do not differ in their sexual orientation from female population norms [151] or from matched female controls [66]. These XY women also are more likely to be exclusively interested in male sexual partners than are XX women with CAH [109], suggesting that the early hormone environment plays a larger direct role than genetic information on the X or Y chromosomes in the development of sexual orientation.

Androgen biosynthesis deficiencies result from enzymatic deficiencies transmitted as autosomal, recessive traits [77,78,120]. They include deficiency in 5-alpha-reductase and deficiency in 17-beta-dehydroxysteroid dehydrogenase. Because 5-alpha-reductase converts testosterone to dihydrotestosterone, deficiency in this enzyme results in low levels of dihydrotestosterone, despite normal to high levels of testosterone [77]. Dihydrotestosterone is necessary for prenatal virilization of the external genitalia, and so XY individuals with this deficiency are born with female-appearing or ambiguous external genitalia. They are usually assigned and reared as girls. At puberty, however, testosterone and other androgens virilize the genitalia, deepen the voice and promote the development of male-typical musculature. A similar outcome is seen with deficiency in the enzyme, 17-beta-dehydroxysteroid

dehydrogenase. This enzyme is needed to produce testosterone from its immediate precursor, androstenedione. Individuals with this deficiency have low levels of both testosterone and dihydrotestosterone, producing a similar physical outcome to that seen for 5-alpha-reductase deficiency. The external genitalia appear to be feminine or ambiguous at birth, the child is typically assigned and reared as a girl, but physical virilization occurs with puberty. In populations where these disorders are common, they sometimes have descriptive names, such as machihembra (first woman, then man) [79] or Turnim Man [64].

Despite their female sex of rearing, 39–64% of individuals with androgen biosynthesis deficiencies change to live as men following physical virilization at puberty, but this outcome does not appear to relate to the degree of genital virilization at birth [24]. Within a single family even, individuals with the same genetic mutation and the same enzymatic deficiency have been found to choose to live as different sexes, one as a man and one as a woman [148]. Information on the sexual activity of those who choose to live as men is far from complete, but in many cases they appear to form sexual partnerships with women [150,154]. Explanations of the post-pubertal gender change, in addition to the effects of the early hormone environment, include possible ambiguity in the sex of rearing, based, for instance, on knowledge that virilization will occur at puberty [64,147]. The decision to change sex also appears to relate in part to cultural factors, or to medical treatment. In the United States and much of Europe, individuals with androgen biosynthesis deficiencies, who have been assigned and reared as girls, often have had their testes removed prior to or early in puberty, to prevent virilization. Individuals treated in this way tend to maintain a female gender identity, and assumedly androphilic sexual orientation [150,154]. This argues against early hormonal determination of their sexual orientation outcome. In other settings, however, virilization at puberty is not prevented, and the individual is faced with a choice of continuing to live as a woman, despite having a masculine physical experience, or changing to live as a man. Added into the mix, in some cultures, there are social and practical advantages of being a male, rather than an infertile female. Thus, androgen exposure at puberty, acting either directly on the brain, or by producing a male body type, or the advantages of being a male in certain cultural groups, also are likely to contribute to some individuals choosing to live as a man, and take a female life partner.

8. Bio-assay of prenatal androgen exposure and sexual orientation: the 2D:4D ratio

Another approach to exploring possible prenatal hormonal influences on sexual orientation has involved correlating physical characteristics that are thought to develop as a result of prenatal androgen exposure with variability in sexual orientation. Much of this research has focused on the ratio of the length of the second digit of the hand to the length of the fourth digit, a ratio that has been termed 2D:4D. This ratio is larger in women than in men and the sex difference is thought to develop under the control of androgenic hormones prenatally [94]. Evidence supporting a relationship to early androgen exposure comes from two studies finding more male-typical 2D:4D in individuals exposed to high levels of androgens prenatally, because of CAH [17,113], but cf [18], and one study finding more female-typical ratios in XY females with CAIS compared to other XY individuals [12].

An initial study relating finger ratios to sexual orientation recruited 720 adults attending public street fairs in the San Francisco area, measured their 2D:4D, and asked them to indicate in an anonymous survey their sex and their sexual orientation [146]. Results showed the expected sex difference in finger ratios on the right hand, with those of 146 heterosexual women being

significantly greater than those of 108 heterosexual men. In addition, right hand 2D:4D was significantly smaller (i.e., more male-typical) in 164 homosexual than in 146 heterosexual women. There was no difference, however, between the 108 heterosexual and 277 homosexual men. In addition, 2D:4D on the left hand did not differ significantly for any of the four groups.

Subsequent research has produced mixed outcomes for the association between 2D:4D and sexual orientation. Some individual studies have suggested that homosexual males show more feminine finger ratios than heterosexual males, but others find no differences or even the opposite result [99]. These inconsistent results may occur because studies of finger ratios are relatively easy to conduct, resulting in publication of many chance findings.

A study commissioned by the British Broadcasting Corporation (BBC) involved over 200,000 individuals who participated online, measuring their own finger lengths and completing questionnaire items about sexual orientation [25]. This study found that 2D:4D on both the right hand and the left hand was more male-typical in 102,499 heterosexual men than in 11,060 homosexual or bisexual men, but no differences were found between 84,417 heterosexual and 9153 homosexual or bisexual women. A meta-analysis that did not include this large online study reported somewhat different findings – an association between a more male-typical finger ratio and non-heterosexual orientation in women, but no relationship in men [57]. Thus, even with very large samples, results for studies of sexual orientation in relation to finger ratios are not completely consistent.

Another type of bio-assay of prenatal androgen exposure is the size of the penis or clitoris, since enlargement of the genital tubercle depends on androgen exposure during early life. Non-heterosexual individuals do not appear to show any obvious alteration in the external genitalia that would suggest that androgen levels were abnormal prenatally. Indeed, there is some evidence that homosexual men have larger penises than heterosexual men [15], a finding that is in the opposite direction of what would be predicted if increasing androgen exposure promoted interest in female sexual partners. However, although both virilization of the external genitalia and neurobehavioral virilization depend on testosterone and its metabolites, there are many ways in which they can be decoupled. For instance, the time period when androgen influences development of the external genitalia is earlier in gestation than the time when most neurobehavioral effects are seen [52]. Also, different co-factors are involved in masculinization of different neural regions [97], and similar differences in the co-factors whose action is required are likely to exist for the genitalia versus whatever neural systems are involved in hormonal influences on sexual orientation. Similarly, testosterone acts on the external genitalia largely following conversion to DHT, but may require conversion to estradiol before exerting some of its neurobehavioral effects. This last difference could even explain the apparent paradox of larger penises, suggesting greater DHT exposure, in homosexual than in heterosexual men. If more of the testosterone these men produce is converted to DHT in the periphery, less could be available for conversion to estradiol in the brain, thus leading to greater masculinization of the external genitalia, but reduced neurobehavioral masculinization. This possibility is highly speculative, however, but the point that genital masculinization and neurobehavioral masculinization need not parallel one another, because of the varied downstream mechanisms involved in testosterone's effects on development, is important.

The issue of differences between the processes involved in neural virilization and those involved in genital virilization also raises the question of whether estrogens play a role in human neurobehavioral masculinization, similar to that documented in rodents. In rodents, manipulations of substances that block conversion of androgens to estrogens or that block estrogen receptors have shown that activation of estrogen receptors is crucial for masculinization in some neural regions and for many sex-related behaviors. Similar manipulations are not possible in humans, and have not been conducted in non-human primates. Based in part on evidence that prenatal treatment with DHT can increase male-typical sex behavior, and reduce female-typical sex behavior, in rhesus macaques, it has been suggested that estrogens might not play a role in neurobehavioral masculinization in primates, including in humans [133]. However, in guinea pigs, DHT has similar masculinising effects to those seen in rhesus macaques [47], but the synthetic estrogen, DES does as well [71,73] Thus, either DHT or estrogens can produce male-typical behavior in guinea pigs. The situation could be similar in primates as well. No studies have reported on sexual behavior following prenatal exposure of non-human primates to DES or other estrogens. However, one study found increased male-typical rough and tumble play in female rhesus macaques exposed to DES during gestation [53]. Surprisingly, rough-and-tumble play is one of the few behaviors that is thought to be masculinized by DHT, but not estrogen, in rats [100]. There appear to be species differences in the role of estrogen in sexual differentiation, and these are not yet completely understood. It appears, however, that a role for estrogen in human sexual differentiation cannot be ruled out, based on research to date.

9. Prenatal stress and sexual orientation

Stress alters production of adrenal hormones, including testosterone and other androgens, raising the question of whether prenatal stress could influence sexual orientation. In rats, stressing pregnant animals increases female-typical sexual behavior in male offspring [138,139]. These effects resemble those seen following reduction of androgen in developing male animals, for example by castration, and the effect of stress on development of male rats has been suggested to occur because it disrupts a prenatal surge of testosterone that occurs in typically-developing males [139–141]. In other rodents, prenatal stress has been found to affect developing females animals in the other direction, impairing female-typical sexual behavior in mice [2] and increasing male-typical courtship behavior in guinea pigs [121]. This could occur because stress stimulates adrenal hormone production. Indeed, stress has been reported to produce high levels of testosterone in pregnant rats [9] and to elevate androgen in fetal mice, both male and female [136].

An early report suggested that prenatal stress might also influence sexual orientation in men. This study interviewed 200 men about stressful events that occurred during their mother's pregnancy with them, and reported that moderately to severely stressful events were recalled by 68% of homosexual men, and by 40% of bisexual men, but by only 6% of heterosexual men [32]. Most of the stressful events had occurred as a consequence of the second world war. A subsequent study, by different authors, of men conceived during the same war did not find increased homosexuality, however [123]. Other studies also have generally found no association between retrospective reports of stress during pregnancy and sexual orientation of male offspring [5,41,145]. Although one study [41] reported a marginally significant difference between homosexual and heterosexual men in retrospective reports of maternal stress during the second trimester of pregnancy, numerous other comparisons were not significant, raising the possibility of a spurious effect. One study of prenatal stress and sexual orientation looked at female offspring, and found that recollections of maternal stress during pregnancy were associated with reduced heterosexual orientation [5]. These findings were echoed in the only prospective study of the effects of prenatal stress on behavioral sexual differentiation. This study found that the amount of stress

reported by mothers during pregnancy correlated with increased male-typical behavior at 3 and one-half years of age in their daughters, but did not correlate with sex-typical behavior in their sons [68]. As will be discussed later, sex-typical childhood behavior is not the same as sexual orientation, but there are some links between the two.

Possible reconciliations of the findings for humans versus rodents involve differences in the adrenal response to stress, and differences in the length of pregnancy. The adrenal response to stress appears to be less dramatic in humans than in rodents [5]. In addition, human gestation and the period of prenatal sexual differentiation is far longer in humans than in rats, appearing to last for approximately 2 days in rats [139], but for months in humans [149]. This additional time may allow feedback mechanisms in the human male fetus to adjust testicular androgen production to compensate for alterations in stress-related adrenal androgen secretion. This explanation would also encompass results suggesting that stress may influence behavioral sexual differentiation in human females but not males, because females do not have testes and so cannot compensate for the altered adrenal androgen release.

10. Sexual orientation and childhood behavior

Boys and girls differ, on average, in their toy, playmate and activity preferences. Boys tend to prefer toys like vehicles, such as cars, trains, trucks and airplanes, and weapons, whereas girls tend to prefer toys like dolls and tea sets [91,115,132]. Sex differences in toy preferences appear in infancy, at least by the age of 12 months [1,19,81,126,130], and they grow larger as children develop into middle childhood [48]. The mode of assessment (e.g., questionnaire versus observation) and the specific toys involved can influence the size of the sex differences, but behavioral observation of toy choices in childhood can show large sex differences for both masculine and feminine toys (1.8–2.0 standard deviations) [70].

In regard to playmate preferences, 80–90% of children's play partners are typically of their own sex [74,90]. This sex difference also grows larger as children grow older; children at age 4.5 years spend about three times as much time with peers of their own than the other sex, whereas at age 6.5 years, they spend 10 times as much time with same sexed as with other sexed peers [92]. Expressed in standard deviation units, the sex difference is very large ($d = 2.3$–5.6) [153]. In regard to activities, boys, like other male mammals, tend to engage in more rough and tumble play, involving overall body contact and playful aggression, than do girls. This sex difference is large for children playing with other children of the same sex ($d = 0.8$–2.1) [28,74,90]. Boys also show higher levels of physical activity, with meta-analytic findings suggesting a sex difference of moderate size ($d = 0.5$), beginning prenatally [35]. Composite measures, that include items assessing toy, playmate and activity preferences also can show large sex differences ($d = 2.7$–3.2) [49,68].

An individual's sexual orientation typically becomes apparent after puberty. However, there is evidence that most men who are not heterosexual recall having been sex-atypical in their childhood behavior, particularly in their sex-typed toy, playmate and activity interests [10,56,61,122]. Taken together, these studies involved over 3000 men in the United States. These results also have been reported cross-culturally, with similar findings for men in Guatemala, Brazil and the Phillipines, to those seen in the United States [143]. Subsequent meta-analysis [6] suggests a large effect size, and this along with additional cross cultural research in men from Independent Samoa [7] strengthens the conclusion that non-heterosexual men recall high levels of cross-gendered behavior in childhood.

Retrospective reports can be compromised by the forgetting of past events or the distortion of past events to accord with the current situation. Therefore, prospective, longitudinal data on the relationship between childhood interests and adult sexual orientation have also been obtained. One study [55] identified 66 boys, ages 4–12 years, who were extremely cross-gendered in their interests. Among these boys, 85% had indicated that they wished to be girls, 70% cross-dressed frequently, 60% frequently played with dress-up female dolls, and 85% had a female peer group. These boys also avoided male-typical activities. They were compared to 55 boys who were conventionally masculine, and who rarely participated in feminine activities. Two thirds of the children in both groups were followed up 15 years later. At this time, 75% of those who had been extremely cross-gendered in childhood were homosexual or bisexual, whereas this was the case for only one of those who had been conventionally masculine [55]. Another long-term, follow up of an initial sample of 55 cross-gendered boys also reported that most were not heterosexual later in life [156]. In this study, 35 of 38 (92%) whose eventual sexual orientation could be determined were not heterosexual.

Findings for childhood toy, playmate and activity preferences for heterosexual versus homosexual women show a similar pattern to those for men. The majority of homosexual women recall a high level of interest in toys, activities and playmates usually preferred by boys, and a low level of interest in those usually preferred by girls [10,56,122]. A meta-analysis suggests that the effect size for women is large ($d = 0.96$), though smaller than that for men ($d = 1.31$) [6]. Also, as for men, cross-cultural data also suggest that non-heterosexual women recall more cross-gendered behavior than heterosexual women, with similar findings from studies in Guatemala, Brazil, the Phillipines and the United States [144]. Two small follow up studies of girls with symptoms of gender identity disorder in childhood also have found reduced heterosexual orientation in adulthood. In the first of these studies, 8 of 25 women (32%) were bisexual or homosexual in fantasy and 6 of 25 (24%) were bisexual or homosexual in behavior [34]. In the second, the majority of 15 girls diagnosed with gender identity disorder in childhood and followed up in adulthood reported being bisexual or homosexual [137]. Among women with CAH also, the amount of gynephilic interest and behavior in adulthood correlates positively and substantially ($r = .51$–$.63$) with recalled male-typical play in childhood [67]. Thus, for these women too, those who show the most cross-gendered childhood interests also show the least likelihood of being heterosexual in adulthood.

11. Hormones and sexual differentiation of childhood behavior

Given the links between childhood interests and adult sexual orientation, understanding the roots of cross gendered interests in childhood could help elucidate the origins of variability in adult sexual orientation.

There is substantial evidence that exposure to androgens prenatally influences children's sex-typical toy, activity and playmate preferences. A consistent research finding, for example, is that girls who were exposed to high levels of testosterone prenatally, because of CAH, show increased male-typical toy preferences, playmate preferences and activity interests [13,30,36,37,59,67,104, 112,115,127,155]. These effects have been seen in studies conducted in a number of different countries in North America and Europe and using various methodologies, including interviews, questionnaires and direct observation of behavior (see Table 1). The results of these studies are consistent with the hypothesis that prenatal exposure to androgens influences the development of children's sex-typical toy, activity and playmate preferences. Evidence from situations where women were prescribed hormones

Table 1
Reports indicating increased male-typical childhood toy, playmate and activity interests in girls with CAH.

Study	Mode of assessment	Location
Ehrhardt et al. [37]	Interview	USA
Ehrhardt and Baker [36]	Interview	USA
Slijper [127]	Questionnaire	The Netherlands
Dittmann et al. [30]	Interview	Germany
Berenbaum and Hines [13]	Observation	USA
Zucker et al. [155]	Retrospective questionnaire	Canada
Nordenstrom et al. [112]	Observation	Sweden
Hines et al. [67]	Retrospective questionnaire	UK
Meyer-Bahlburg et al. [104]	Questionnaire and observation	USA
Hall et al. [59]	Questionnaire	UK
Pasterski et al. [115]	Observation	UK and USA

during pregnancy for medical reasons also support a role for androgens prenatally in the development of children's sex-typed interests. Children whose mothers took androgenic progestins during pregnancy show increased male-typical or decreased female-typical behavior, and those whose mothers took anti-androgenic progestins show the opposite outcome [38,40].

Girls with CAH have a disorder, and they, as well as girls whose mothers took androgenic progestins while pregnant, typically are born with virilized genitalia, leading to suggestions that physical virilization, or other aspects of the CAH disorder, could cause increased male-typical behavior [42,117]. These suggestions have been addressed by showing that normal variability in androgen exposure prenatally, as well as elevation in androgens caused by CAH, is associated with normal variability in sex-typed toy, activity and playmate preferences postnatally. One study looked at maternal testosterone levels during pregnancy in relation to subsequent, sex-typical behavior in offspring at age 3.5 years. This study involved a population sample of over 9000 children from which extreme groups of masculine and feminine children were selected and compared, along with a random sample of control children. The extremely masculine girls had mothers with the highest levels of testosterone during pregnancy, followed by the random sample, and then the mothers of the extremely feminine girls, and the data showed a significant linear relationship between maternal testosterone and male-typical behavior in female offspring [72]. A similar relationship was not seen in boys. Testosterone levels were similar in women carrying male and female fetuses [72], arguing against testosterone passing from the fetus to the mother. Nor is it necessary that testosterone passes from the mother to the fetus, although this could occur if testosterone levels are sufficiently high. The most likely explanation for the results, however, involves genetic and endocrinological similarity between mothers and daughters. Testosterone in maternal blood during pregnancy correlates positively and substantially with testosterone in fetal blood sampled at the same time ($r = 0.414$) [46]. Testosterone production is determined, in part, by genetic factors, with heritability estimates ranging from 40% to 60% [60,128]. In addition, testosterone correlates positively and significantly in mothers and daughters, but not in mothers and sons [60], and this fits with the different sources of testosterone production in males versus females. Both pregnant women and their daughters produce testosterone from their adrenal glands and ovaries, whereas, for sons, the testes are the major source of testosterone. Thus, female fetuses whose mothers have high levels of testosterone tend to produce high levels of testosterone themselves, because of genetic resemblance, resulting in a relationship between maternal testosterone and sex-typed behavior in girls, and the lack of a similar relationship in boys.

A second study involving normal variability related testosterone measured prenatally in amniotic fluid to male-typical toy, activity and playmate preferences in childhood, and found significant relationships in boys and in girls [4]. These results also show the hypothesized relationship between prenatal testosterone and sex-typed behavior in healthy individuals with no genital abnormalities. Thus, they provide convergent evidence, with the data from studies of CAH and of maternal testosterone, of influences of prenatal testosterone exposure on subsequent sex-typical behavior in childhood. Although two other similar studies found insignificant correlations between testosterone in amniotic fluid and childhood sex-typed behavior [87,135], these negative results could have resulted from small sample sizes and from the use of relatively insensitive behavioral measures.

12. Pathways to sexual orientation

The factors leading to individual variability in most human psychological and behavioral characteristics are rarely singular. In most cases, several types of factors combine to produce a particular outcome. In addition, a number of different pathways can lead to a common outcome. Sexual orientation outcomes are likely to be similarly determined by multiple factors, and to have more than one multi-factored pathway leading to an outcome, such as androphilic orientation, gynephilic orientation or bisexual orientation.

This article addressed the question of whether gonadal steroid exposure during prenatal development is one of the factors, in at least one of the pathways, that lead to variability in sexual orientation outcomes. Based on the compelling evidence that prenatal testosterone exposure influences children's sex-typical play behavior, on the well-established links between childhood play interests and adult sexual orientation, and on the evidence showing altered sexual orientation in women exposed to high levels of androgens prenatally, because of CAH, the answer appears to be "yes".

It is worth discussing the reason for the link between sex-atypical toy, playmate and activity interests in childhood and sex-atypical sexual orientation in adulthood. One suggestion is that the two are causally related, with children who avoid same sex playmates and their activities subsequently finding these children sexually arousing, because "exotic becomes erotic" [11]. One problem with this theory is that it would seem to suggest that other sources of unfamiliarity, such as racial differences, should also lead to increased sexual attraction, and this does not seem to be the case. Although Bem [11] suggests that the effect is confined to gender unfamiliarity, rather than to racial or other aspects of unfamiliarity, because there is an optimal level of unfamiliarity, this explanation is unsatisfying, because the optimal level cannot be specified. In addition, different non-heterosexual individuals would be likely to have spent different amounts of time in childhood with individuals of the same sex, thus necessitating a different optimal level from one individual to the next. A more parsimonious explanation, and the one I think more likely, is that the prenatal hormone environment influences both of these sex-related characteristics, childhood toy, playmate and activity interests and sexual orientation, directly, by acting during early development on the neural systems that regulate them.

It also is noteworthy that, despite the evidence that many women exposed to high levels of androgens prenatally because of CAH are not heterosexual, most, even many of those with the most severe form of the disorder, are exclusively or almost exclusively heterosexual. Similarly, although many homosexual men and women recall cross-gendered childhood interests, this is not universal. In addition, most people who are not heterosexual have no evidence of prenatal hormone abnormality. It is possible that these individuals have hormonal differences in circumscribed brain

regions, or abnormalities downstream from testosterone itself, e.g., in PGE_2, but such possibilities are highly speculative. In addition, factors other than the early hormone environment are likely to be important for the development of sexual orientation. There is at present very little information, however, as to exactly what these factors might be. Other articles in this issue suggest genetic involvement, given the heritability of sexual orientation, as suggested, for example, by twin studies. Little or no information is available, however, as to the specific genetic factors involved, and it is possible that the genetic factors could act through hormones, by, to give just two of many possibilities, influencing testosterone synthesis or levels of androgen receptors. Similarly, and also as detailed elsewhere in this issue, for males, the number of older brothers has been linked to sexual orientation and interpreted to suggest that maternal factors influence the developing male fetus. Could these factors also involve hormonal mechanisms?

13. Directions for future research

The apparent differences in sexual orientation outcomes for individuals with the same androgen deficiency disorder, and the apparent importance of virilization at puberty or of cultural factors in determining sexual orientation in these syndromes is intriguing. It may be that one's physical appearance plays an important role in sexual orientation, at least for some people. For instance, an ambiguous early hormone environment may make it easier to follow a sexual orientation path that accords with one's physical appearance and with the way that society reacts to that appearance. A first step in exploring these possibilities would be to obtain more rigorous assessments of sexual orientation outcomes in individuals with androgen biosynthesis disorders. Currently, information is limited and sexual orientation, for example, in fantasy or imagery, is largely unknown.

A second outstanding question regards sexual orientation in women with non-classical CAH. As mentioned previously, this form of CAH is thought to involve only postnatal hormonal abnormality. In the only large scale study of sexual orientation in this group to date, however, non-classical CAH appears to be associated with a significant decrease in heterosexual orientation [103]. Although this outcome was not replicated in a second study [44], the second study had a small sample and assessed orientation as only heterosexual, homosexual or bisexual. If the initial result is replicated using larger samples, and sensitive assessment of sexual orientation in both fantasy or imagery and behavior, it could suggest a postnatal influence of androgen exposure, perhaps at puberty, on sexual orientation in some individuals (see [65] and Berenbaum and Beltz in this issue for additional discussion of this possibility). Although this would seem unlikely, particularly for non-heterosexual individuals who recall cross-gendered childhoods, it might provide an alternative pathway for those with conventional childhoods. The hormonal changes of puberty produce permanent physical changes, for example, in penis and breast size, and recent research in rodents suggests that hormonal changes at puberty have some enduring, organizational-type influences on neurobehavioral development related to reproduction [124]. Alternatively, non-classical CAH may involve prenatal androgen excess, similar to, though less marked than that associated with classical CAH, and this possibility also merits investigation.

A third focus for future research might be on identifying additional ways to assess the early hormone environment in healthy individuals. The literally hundreds of studies that have been published in the past dozen or so years relating the 2D:4D finger ratio to various behaviors and other characteristics that show sex differences provide evidence of interest in such measures.

Unfortunately, studies of 2D:4D have produced inconsistent results, even in large samples. Another approach has been to look at testosterone in amniotic fluid samples, but this approach is limited to women referred for amniocentesis for clinical reasons, so not necessarily representative of the general population. In addition, only one sample of amniotic fluid at a single timepoint, usually uncontrolled for time of day, is available. One report on extreme groups from a large population sample suggests that maternal testosterone during pregnancy might provide a window into the hormone environment of the female fetus [72]. Although maternal hormones appear far from fetal hormones, there appears to be a substantial correlation between the two [46], probably because of genetic relatedness [60,128]. One advantage of maternal samples, either of blood or of saliva, is that they can be obtained repeatedly in the population at large. This potential window into hormones during early development deserves additional attention.

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