Sexual differentiation is a stepwise process starting with a difference of the sex chromosomes (XX for females, XY for males). The embryo starts off with two basic pairs of reproductive structures, the Müllerian ducts and the Wolffian ducts. A gene located on the Y chromosome (SRY) induces the development of the testis. A few weeks after conception, the testes will start to produce testosterone and Müller Inhibiting Substance (MIS). These hormones of the testes direct male development. The Wolffian ducts develop into male internal reproductive organs, and the MIS, produced by the Sertoli cells of the developing testes, causes the Müllerian ducts to regress. In the absence of a Y chromosome (and therefore testes and androgens), ovaries will develop. The Wolffian ducts will regress and the internal sex organs will develop along the female line, the default route. The external genitals also develop from identical structures. In males, testosterone and its derivative dihydrotestosterone (DHT) direct the genital tubercle to become the penis and the genital swellings fuse to form the scrotum, whereas in females, in the absence of testosterone, these structures become a clitoris and a labia.

Apart from the sexual differentiation of the genitalia, sex hormones in the prenatal environment influence the differentiation of the brain into male or female. Pre- and early neonatal exposure of the brain to sex hormones leads to permanent changes in the nervous system. These effects are referred to as organizational effects. From vertebrate models we learn that the steroid hormone testosterone accounts for the majority of the known sex differences in neural structure and behavior. In lower animals, the presence or absence of testosterone at the time of a critical period of brain sexual differentiation influences the morphology of certain brain nuclei. Like its influence on the development of the genitalia, the presence of testosterone leads to male sexual differentiation of the brain and results in male-typical behavior, while a
female brain and female-typical behavior are found to be the outcome of the absence of testosterone. When the testes are formed, they begin to produce testosterone and from this moment on there is a sex difference in testosterone concentrations between male and female fetuses. Through its effects on neurogenesis, cell migration, cell death, and the differentiation of neural circuits, testosterone has its effects on neuronal organization.

**Influence of prenatal hormones on male gender development in humans: evidence from non-clinical samples**

Gonadal hormones are also thought to influence the sexual differentiation of brain and behavior in humans, but the exact mechanisms and timing remain unclear. Early in life, sex differences are observed in play behavior and preferences. An approach to study the effects of prenatal testosterone on gender development is to relate hormonal levels in maternal serum or amniotic fluid to variations in subsequent gender-related (play) behavior in non-clinical samples. Maternal testosterone predicted the amount of male-typical behavior in daughters, as measured by parent questionnaires. Amniotic testosterone was found to be related to male-typical play behavior, as assessed by maternal reports of childhood sex-typed activities in male as well as female offspring. However, other studies did not find support for the hypothesized relationship between prenatal testosterone exposure and postnatal gender-related play behavior.

Another research paradigm to study the influence of prenatal brain exposure to sex hormones comes from the study of opposite-vs. same-sex twin pairs. It is assumed that fetal androgens may be transferred from the male to the female fetus and that the female twin might thus be androgenized by her male co-twin. However, results of such studies have been found to be inconsistent.

**Early cognitive gender development**

From cognitive developmental studies we know that learning about being a boy or a girl starts in infancy. Babies as young as 9 months are already able to visually discriminate between the sexes. The ability to verbally label the sexes comes later, at around 28 months. As toddlers are often hardly aware of genital differences, they use hairstyle and clothing as a criterion for classification.

With regard to the concept of gender, children first learn to identify their own and others’ sex (gender labeling). Next, they learn that gender is stable over time (gender stability). Finally, they learn that superficial changes in appearance or activities (a boy does not become a girl overnight if he puts on a wig or plays with Barbie dolls) does not change one’s gender. This is the last stage of gender constancy (gender consistency). This last phase is reached between 5–7 years, but long before that age, children appear to have knowledge about gender stereotypes (for an overview see Ruble *et al.* 2006). For instance, 3-year-old children, who saw videotaped infants labeled male, rated these infants as “big,” “mad,” “fast,” “strong,” “loud,” “smart,” and “hard.” When labeled female, they were rated as “small,” “scared,” “slow,” “weak,” “quiet,” “dumb,” and “soft”. Three-year-olds also believe that “boys hit people.” Gender stereotype knowledge increases rapidly after 3 years of age and appears to develop throughout childhood. Once established,
gender stereotypes influence the way new information is processed. Children remember stereotype consistent information better than inconsistent information, and even distort inconsistent information. For instance, when a picture is shown to them of a woman flying an airplane, they may either report having seen a man flying the airplane or a woman doing something else, such as cooking.

According to some cognitively oriented theorists, children need only basic information rather than extensive knowledge about gender to further develop gender role behavior. For instance, children prefer same-sex toys, imitate same-sex models, and reward peers for gender-appropriate behavior before they reach complete gender constancy. Therefore, a complete understanding of gender is perhaps not important in the very early stages of gender development.

Gender development is a process that not only involves cognitive aspects but also involves affective meanings. As soon as a child identifies with one of the sexes, these values will affect their self-perception and self-concept. For instance, boys are usually proud of being a boy and look somewhat down on girls.

Gender segregation
At very early ages children become interested in same-sex playmates. Boys like other boys better than girls and spend a fair amount of time in the company of other boys. Changing this peer preference appears to be difficult.

Children thus spend an important part of their time in all-male or all-female groups. Boys tend to play in larger groups, play in more public places and with less proximity to adults, and play rougher and with more body contact. Boys fight more and their social interaction is oriented more toward issues of dominance. Girls’ groups are less hierarchically organized and their friendships are more intense. Girls appear to use language to create and maintain relationships, to criticize others in acceptable ways, and to interpret accurately the speech of other girls. In boys, speech is used to attract and maintain an audience, to assert one’s position of dominance, and to assert oneself when others have the floor. So gender segregation has far-reaching consequences for children's social development and friendships.

The influence of the environment on gender development
Children also learn about gender by observation of role models and by differential treatment. This differential treatment may be more or less direct (e.g. playing different games with boys than with girls) or be more subtle or indirect (e.g. blue and pink clothing). An immense body of literature supports the notion that parents, other adults, teachers, peers, and the media are gender-socializing agents. For instance, mothers talk more to daughters than to sons, teachers praise and criticize boys more than girls, and peers reinforce same-sex and punish cross-sex behavior. In experiments in which the actual sex of an infant is unknown, adults even interact differently with children labeled as boys than with children labeled as girls.

Adults and children are not just influencing gender development by their reinforcement of behaviors. As role models, parents and peers also shape children's gender attitudes and behaviors. Furthermore, gender development seems to be strongly influenced by the media. This was nicely illustrated by an older study among children living in a Canadian town unable to receive television. Before television was introduced, they were less traditional than a control group. Two years later their attitudes had changed dramatically in the more traditional direction.

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Besides biological factors, such as prenatal exposure to testosterone, environmental and psychological factors also play a role in male gender development.
Gender development: sex hormones and the brain after puberty

Later in life, neural circuits and behavioral patterns are activated by changing levels of sex hormones. An example of these so-called activating effects is the stimulation of the already sexually differentiated nervous system by gonadal hormones during puberty. Because steroid-dependent organization of brain and behavior also takes place during adolescence, it has been suggested that these activating effects should also be characterized as organizational effects. The timeframe for organizational effects may not be limited to prenatal and early neonatal periods, but may also include puberty and adolescence. Steroid dependent organization during puberty implies that certain adult sex-typical behaviors are expressed because pubertal hormones first have organized neural circuits in the developing adolescent brain and that these circuits are subsequently activated by gonadal hormones. One example of structural changes that may be the result of pubertal hormone changes is the white matter volume. This increases faster and reaches a bigger overall volume in boys than in girls during puberty and it is thought that white matter volume might be related to the activity of the AR. With regard to brain tissue in adulthood, sexual dimorphism is found for gray and white matter, but the white matter difference is more pronounced, with men having larger white matter volumes than women. Regionally, larger volumes of gray matter are detected in women than in men.

Other sex differences in brain and behavior in adulthood may also be related to the effects of sex hormones. Subcortically, in the hypothalamus, sex differences are observed in the interstitial nuclei of the anterior hypothalamus (INAH-1, INAH-2, INAH-3) and the central portion of the bed nucleus of the stria terminalis, with larger volumes in men than in women. These sex differences in the hypothalamus are thought to underlie sex differences in gender identity, reproduction, and sexual orientation.

Gender-related cognitive functioning has been related to size and shape of the corpus callosum. Sex differences have been reported for the corpus callosum, but there is disagreement about the direction of the sex effect and some studies failed to detect such an effect. Men do show more morphological asymmetry than women and appear to have a somewhat more lateralized brain with left hemisphere dominance for language processing and right hemisphere dominance for spatial processing.

Regions with developmentally high densities of estrogen and ARs show greater sexual dimorphism. For example, the amygdala has a larger volume in males. Sex differences in the amygdala's response have been mentioned as factors to explain sex differences in the prevalence of psychiatric disorders. For instance, depression is less common in men than in women and is associated with sex differences in the role of the amygdala in emotional memory.

Men and women also differ in the occurrence of other psychiatric disorders. Schizophrenia, attention deficit hyperactivity disorder, and autism primarily hamper men (for an overview see Bao & Swaab 2010), whereas eating- and anxiety disorders are more prevalent in women. Sex ratios for neurological disorders differ as well, with Rett syndrome (non-existent in men) and Kleine-Levin syndrome (non-existent in women) as extremes. Finally, personality characteristics also show sex differences. In general, physical aggression appears to be higher in men, whereas empathy has been found to be higher in women.

Genetic studies

Independent of the role of hormones, other biological factors, such as genes, may also influence gender development. Even before the production of gonadal hormones, genes may directly affect brain sexual differentiation. Evidence for the role of genetic factors in sex differences in behavioral traits has been found for play behavior and
aggression. For a review of the evidence for direct genetic effects on sex differences in brain and behavior we refer to Ngun et al. 2011.

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**Gender identity:** a person's sense of self as being male or female.
**Gender role:** behaviors, attitudes, and personality traits that a society, in a given culture and historical period, designates as more typical of the male or female social role.
**Disorders of Sex Development (DSD)** (previously referred to as intersex conditions): congenital conditions in which the development of chromosomal, gonadal, and/or anatomical sex are not entirely male or female.
**Gender dysphoria:** is the distress resulting from conflicting gender identity and gender of assignment.
**Transsexualism or gender identity disorder:** extreme end of the spectrum of gender dysphoric conditions, usually characterized by a pursuit of sex reassignment.

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**A typical development of gender identity and gender role behavior**

Research on factors that influence typical gender development generally focuses on gender role or gender related behavior. We have already seen that in non-clinical groups, prenatal exposure to higher levels of androgens may lead to more masculine behavior. Because gender identity usually develops in accordance with an uneventful sexual differentiation, it is difficult to study factors that may influence gender identity development in typically developing individuals. However, studies in individuals with atypical prenatal hormonal levels or individuals that have a gender identity that is not in accordance with their natal sex could help in elucidating the mechanisms underlying gender identity development. Three groups are of interest: children of mothers who took medication during their pregnancies that might have influenced their children's gender development, individuals with disorders of sex development, and transgender individuals.

**Intoxications during pregnancy**

Daughters of mothers who took diethylstilbestrol (DES), a synthetic estrogen that masculinizes and defeminizes brains and behavior in female rodents, have been found to show higher rates of homosexual imagery or homosexuality than controls, but no masculine gender identity. Effects of exogenous hormones on male behavior and interests are less clear and often conflicting.

In a study among, adults who had prenatally been exposed to phenobarbital- and phenytoin, known to influence sex steroid metabolism, it was found that the individuals as a group did not differ with respect to gender role behavior, but that higher numbers of prenatally exposed subjects reported current or past gender variant behavior and/or gender dysphoria. Gender dysphoria is the distress resulting from conflicting gender identity and gender of assignment. In a group of 147 subjects, there were also 3 transsexuals. This is a remarkably high rate given the rarity of transsexualism.

**Disorders of Sex Development (DSD)**

**Gender development in individuals with CAH**

Congenital adrenal hyperplasia (CAH) exposes female fetuses to elevated testosterone levels. This condition is extensively studied to infer the relationship between prenatal hormones and postnatal gender development. These women, who are born with more or less virilized external genitalia, are generally treated early in life to normalize hormone levels and often undergo surgery to feminize
their genitalia. Girls with CAH generally show increased male-typical play behavior. Masculine gender role behavior also appears to be common in women with CAH across the lifespan. In women with CAH, a dose-response correlation has been found; with the more seriously affected “salt-losing” women showing more masculine behavior than the less affected “simple-virilizing” women.

In contrast, women with CAH, who were raised as females, mostly have feminine gender identities. However, these women show a less strong female identification, elevated levels of gender discomfort, and even gender dysphoria (∼5%) than non-DSD women.

Gender development and 5α-reductase-2 deficiency (5α-RD-2) and 17β-hydroxysteroid dehydrogenase-3 deficiency (17β-HSD-3)

Children with 5α-RD-2 have an enzyme defect that prenatally blocks the conversion of testosterone into dihydrotestosterone. Consequently they are born with external genitals that are female in appearance. They are usually raised as girls and seem to have a female gender identity, but, if the condition is not discovered in childhood, these children develop male sex characteristics in puberty: growth of their “clitoris” and scrotum, lowering of the voice, beard growth, masculine muscle development, and masculine body fat distribution. After puberty, many of these youngsters start living as males and develop a sexual attraction toward females. These transitions have been primarily documented in non-Western cultures. When raised as boys, these children have a male identity and behave like boys.

Another condition affecting testosterone biosynthesis, which might lead to impaired virilization in male infants, but excessive virilization when these children become adolescents, is 17β-HSD-3. Gender transitions in 46,XY children with 17β-HSD-3 raised as girls have also been reported. However, such changes did not happen in all affected individuals. De Vries and colleagues (2007) reviewed the literature on gender identity outcome and DSD and found that 59% of the female-raised 5α-RD-2 individuals (69 of 117), and 39% of the 17β-HSD-3 individuals (20 of 51), all above age 12, had gender dysphoria to the extent that they chose to live as males later in life.

Gender development and CAIS/PAIS

Individuals with complete androgen insensitivity syndrome (CAIS), who are raised as girls, are described as very feminine in their gender role behavior, although there may be more variability in their behavior than has long been assumed. They have a female gender identity and in the review by de Vries et al. (2007), none of the women with CAIS reported suffering from gender dysphoria or made a gender transition. But in the partial form of this condition, partial androgen insensitivity syndrome (PAIS), another picture emerges. In female-raised individuals, 11% were gender dysphoric or changed gender (5 of 46). In the male-raised group, this percentage was even higher, where 14% were gender dysphoric or changed gender (5 of 35).

Gender development and ablato penis

A famous case of male identical twins is illustrative in this nature/nurture debate as well. One of the boys lost his penis due to a circumcision accident. The parents were advised to re-assign the child to the female gender and raise him as a girl. Early reports showed that, in contrast to the twin brother, the reassigned child seemed to develop as a “real girl,” despite the fact that she had many tomboyish traits. Later, the boy became increasingly unhappy as a girl, and as an adolescent he reassumed the male role. He married and became the stepfather of children. The easily drawn conclusion from this case, that prenatal hormones determine gender identity, seems to be premature, however. In a review reporting on 6 more cases of ablato penis, the majority lived as females without gender dysphoria.
Male gender role behavior in female-raised children should not be mistaken for a male gender identity.

Concluding remarks

Many parents of children with DSD are concerned about their gender. Some parents are ignorant about the sex of their child for some time. Children with DSD may be ill at birth and may need medical interventions. In addition, the conditions that have been studied vary widely. Levels of prenatal hormones, and timing and duration of the exposure differ between conditions or between individuals with similar conditions and are usually unknown. Therefore, extrapolating the above findings, on atypical gender role development to normal development, has obvious limitations. It is clear from the results of studies of DSD individuals, that a distinction between gender-role behavior and gender identity has to be made. These study results support the aforementioned findings in non-clinical samples, indicating a relationship between prenatal androgenization and masculine behavior. Whether and to what extent prenatal androgen brain exposure results in a male gender identity is less clear.

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Prenatal testosterone appears to influence the development of masculine gender role behavior more than the development of a male gender identity.

Transsexuals

Transsexuals have a gender identity that is inconsistent with their natal sex and strongly desire to live in accordance with their gender identity. A complete cross-gender identity may be present from very early ages on. Parents often report that their sons never showed male-typical behavior. Why such young boys identify with girls and want to behave like girls is still an enigma. A supposed discrepancy between genital differentiation on the one hand and hormone induced brain sexual differentiation on the other has been invoked as an explanation for the phenomenon. Because it is impossible to determine prenatal hormone levels in adulthood, post-mortem brain studies, cognitive, handedness and imaging studies are employed to investigate whether the brains of transsexuals resemble those of their natal sex or of their gender identity.

Post-mortem studies

Postmortem studies into the brain material of transsexuals revealed a sex reversal in volume and neuron number in the central portion of the bed nucleus of the stria terminalis and the interstitial nucleus 3 of the anterior hypothalamus in male-to-female transsexuals (MtFs) and a female-to-male (FtM) transsexual. Because all subjects had received hormone therapy, it remains unclear if the differences should be ascribed to this treatment. However, non-transsexual males, who had taken estrogens for medical reasons, did not show a smaller central portion of the bed nucleus of the stria terminalis.

Luteinizing hormone (LH) regulation

Based on the assumption that neuroendocrine regulation of LH is a reliable indicator of the sexual differentiation of the brain, it was postulated that MtFs, like females, would show a rise in LH levels after estrogen stimulation (estrogen positive feedback effect) as a consequence of prenatal exposure to imbalanced sex steroid levels. The opposite was expected to occur in FtMs. However, studies with thorough methodology found no support for a sex reversal in the neuroendocrine regulation of LH in transsexuals.
Cognitive studies
A relationship between prenatal hormonal influences, on the one hand, and sexual differentiation of the brain on the other hand, might be reflected in sex-related cognitive abilities and functional cerebral asymmetry. If a cross-gender identity is (partially) determined by prenatal brain exposure to unusual sex hormone levels, one would expect that transsexuals would resemble their desired sex more than their sex of birth with regard to sex-related cognitive abilities. FtMs would have more male-typical outcomes and MtFs more female-typical outcomes.

In studies examining IQ and verbal/spatial subtests, some samples of individuals with gender identity disorder (GID) show a pattern of cognitive functioning that is not in line with the natal sex. However, the results are too inconsistent to draw definite conclusions. Moreover, the IQ scales are not developed to measure constructs that show large sex differences. Therefore attempts have been made to compare transsexual and non-transsexual samples with other instruments. To reliably study organizing effects of sex hormones on cognitive functioning, one should study untreated transsexuals, that is before any hormonal treatment and surgical steps. In this way differences between transsexuals and controls sharing their natal sex cannot be attributed to the treatment.

Only a few studies examined these constructs in untreated transsexuals. Female patterns of cognitive functioning and functional cerebral asymmetry in MtFs were reported. MtFs showed less functional cerebral asymmetry when processing auditory verbal stimuli and they performed better on a verbal memory test than male controls. In addition, the predicted pattern of gender-atypical cognitive functioning on a verbal memory test was found in FtMs. In another study, untreated MtFs and FtMs scored between male and female controls on male and female favoring tasks. However, still others found a cognitive pattern in untreated individuals with GID that was consistent with that of their natal sex.

Handedness studies
Whether handedness differs between individuals with a GID diagnosis and controls has been investigated both in children and adults. In these studies, the assumption was that prenatal hormone brain exposure explained both the GID and the non-right-handedness. More left-handedness was indeed found in boys with GID than in a clinical control group and in 3 population studies of non-referred boys. Elevated percentages of non-right-handedness in adult MtFs and FtMs were also found.

Imaging studies
Nowadays, imaging techniques are used to investigate whether there is a sex reversal in structure or in functioning of the brains of transsexuals. Diffusion Tensor Imaging is a technique to display the white matter microstructure pattern. Untreated FtMs show a white matter microstructure pattern that resembles their gender identity more closely than their natal sex, and the pattern of untreated MtFs falls in between that of men and women. Magnetic Resonance Imaging (MRI) in untreated MtFs shows that their gray matter volumes are mostly consistent with men (their natal sex), but that the gray matter volume of the putamen was feminized. In addition, cerebral activation patterns in transsexuals prior to treatment seem to share more features with those of the experienced gender than those of their natal sex. This was observed using Positron Emission Tomography (PET) during the processing of pheromones and using functional MRI while viewing erotic film excerpts. Finally, differences have been found within the cortical network engaged in mental rotation between MtFs (prior to as well as during hormonal treatment) and control males. Also, a study in transsexuals under cross-sex hormone
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treatment showed a difference in parietal activation during mental rotation between MtFs and control men, which might also be a result of *a priori* differences between the MtFs and those sharing their natal sex (male controls).

**Summary and conclusion**

From studies in both clinical and non-clinical samples, it seems safe to infer that prenatal exposure to androgens influence certain male gender role behaviors. Not only 46,XY but also 46,XX individuals who are exposed to high levels of testosterone, exhibit behaviors that are typically attributed to males. With regard to gender identity, we cannot draw similar conclusions with the same level of confidence. Elevated percentages of gender dysphoria have been found in 46,XX individuals with known exposure to atypical levels of androgens, but there is not a one-to-one relationship between such exposure and gender identity problems. Also, in individuals with a gender identity that does not correspond to their natal sex, there are indications of exposure to atypical levels of sex hormones. However, these results again do not point to a one-to-one relationship between gender identity and prenatal sex hormone levels. With regard to male development, it seems likely, on the basis of the current evidence, that sex hormones, androgens in particular, lay important groundwork for gender development. Prenatal androgens result in male-appearing genitals at birth. As a result, the environment will consider the child as a boy and treat him likewise. Prenatal brain exposure to androgens also results in various male-typical behaviors. The developing boy will consider (male genitals) and label himself (cognitive development) as a boy, choose male models and increasingly create his own social environment. If all these factors (body/genitals, perception of the environment, self-perception, behaviors, and preferences) work in accordance with each other and reinforce each other, there seems to be no other possible outcome in adulthood than a firmly established male gender identity and corresponding male behavior patterns, including sexuality. However, when some of these elements work for some reason against a male pathway, it is likely that the adult will have a gender variant identity and/or show gender variant behaviors and preferences.

**Selected bibliography**


