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REVIEW ARTICLE

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The role of steroid hormones in the sexual differentiation of the human brain

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Abstract

Widespread sex differences in human brain structure and function have been reported. Research on animal models has demonstrated that sex differences in brain and behavior are induced by steroid hormones during specific, hormone sensitive, developmental periods. It was shown that typical male neural and behavioral characteristics develop under the influence of testosterone, mostly acting during perinatal development. By contrast, typical female neural and behavioral characteristics may actually develop under the influence of estradiol during a specific prepubertal period. This review provides an overview of our current knowledge on the role of steroid hormones in the sexual differentiation of the human brain. Both clinical and neuroimaging data obtained in patients with altered androgen levels/actions (i.e., congenital adrenal hyperplasia or complete androgen insensitivity syndrome [CAIS]), point to an important role of (prenatal) androgens in inducing typical male neural and psychosexual characteristics in humans. In contrast to rodents, there appears to be no obvious role for estrogens in masculinizing the human brain. Furthermore, data from CAIS also suggest a contribution of sex chromosome genes to the development of the human brain. The final part of this review is dedicated to a brief discussion of gender incongruence, also known as gender dysphoria, which has been associated with an altered or less pronounced sexual differentiation of the brain.

KEYWORDS

androgens, gender, hypothalamus, neuroimaging, puberty

1 | INTRODUCTION

Sex differences in the human brain and how these might relate to sex differences in cognitive functioning have always been a topic of strong interest. In particular, there is an ongoing debate on whether sex differences in brain and behavior are the result of specific biological processes during development ("nature") or whether they are the result of the social environment ("nurture"). For example, do boys prefer to play with cars and girls with dolls because they have been encouraged to do so by their social environment or are there some innate factors that have determined such sex-typed toy preferences? At present, there is strong evidence that biological factors and, in particular steroid hormones, play an important role in organizing the developing brain in either a typical male or female direction and that sex differences can already be present at birth or are expressed in early childhood. This review presents a short overview of our current knowledge on the origin of sex differences in the human brain. Most of our current knowledge on this topic has been derived from analyzing brain structure and function in patients with disorders of sex development, also known as differences in sexual development (DSD). Finally, the potential role of pubertal hormones in neurodevelopment is discussed in light of the proposed treatment for adolescents diagnosed with gender incongruence.

2 | SEXUAL DIFFERENTIATION OF THE HUMAN BRAIN

In mammals, including humans, the developing organism has the potential to become either male or female. The presence of the SRY gene on the Y chromosome will induce the formation of testes from the undifferentiated gonads in males,[1] whereas, in its absence, ovaries will develop. The newly differentiated testes will produce testosterone, which promotes the development of the typical male genital structures. By contrast, typical female genital structures appear to develop without any apparent hormonal input from the ovaries. This process of gonadal and genital differentiation takes primarily place within the first 12 weeks after conception.

The sexual differentiation of the human brain is generally believed to start around the 8th week after conception and lasting at least until the 24th week. This has been based on the observation that testosterone levels are higher in male than female fetuses during this particular prenatal period.[2,3] However, the sexual differentiation of the brain might continue after birth because the first postnatal months are marked by a surge in testosterone in boys peaking at around 3 months after birth, and increased estradiol levels in girls, which decrease more gradually during the second year of life.[4,5] This period is commonly referred to as "mini-puberty".[5]

Research on animal models has been crucial in framing the questions about the origins of sex differences in the human brain. These animal studies have convincingly shown that steroid hormones are key in inducing sex differences in the brain and behavior. Thus, testosterone induces typical male neural and behavioral characteristics during perinatal development, whereas, in its absence, typical female neural and behavioral characteristics develop (i.e., by default). However, it should be noted that there is some experimental evidence indicating that estradiol is actually required for full typical female development.[6] In addition to steroid hormones, evidence has been emerging indicating that genes on the sex chromosomes might also contribute to the sexual differentiation of the brain.[7] Transgenic mouse studies in which the *Sry* gene was deleted from the Y chromosome and inserted into an autosome, making it possible to differentiate between direct genetic and hormonal effects on the brain and behavior, showed that some sex differences actually depend on the presence of the Y-chromosome.[8] Furthermore, transgenic mouse models showed that the number of X chromosomes might also contribute to sex differences in disease phenotypes.[9]

For obvious ethical reasons, analyzing the role of steroid hormones and/or sex chromosome genes in the sexual differentiation of the human brain is more complicated because one cannot manipulate hormone levels or "knockout" genes as in rodent models. Postmortem studies have been useful for investigating structural sex differences in the human brain, also in relation to sexual orientation and gender identity, although they do not shed much light on the mechanisms contributing to these sex differences. So, at present, most of our knowledge on the potential mechanisms contributing to the sexual differentiation of the human brain has been derived from studying DSDs. Such studies refer to congenital conditions in which the development of chromosomal, gonadal or anatomical sex is atypical.[10,11] Although many DSDs are not well studied in the context of the sexual differentiation of the brain, some of them have provided a unique opportunity to assess the different players involved in sexual differentiation, such as congenital adrenal hyperplasia (CAH), sex chromosome aneuploidies (SCAs) (e.g., Turner syndrome [TS], Klinefelter syndrome [KS]) and complete androgen insensitivity syndrome (CAIS). The findings obtained particularly in CAH and CAIS are reviewed below.

3 | THE ROLE OF PRENATAL ANDROGENS IN THE SEXUAL DIFFERENTIATION OF THE HUMAN BRAIN

In rodents, there is abundant evidence that the masculinization of the brain (i.e., the development of typical male neural and behavioral characteristics) is actually mediated by estradiol, locally produced from testosterone by the enzyme aromatase. This is most evident in rats, which comprise the most commonly used animal model for investigating the sexual differentiation of the brain and behavior during the last century. By contrast, in non-human primate species, little evidence exists for such a masculinizing role of estradiol.[12] For example, female rhesus monkeys that had received high doses of androgens during prenatal development showed masculinized juvenile and adult sexual behaviors, as well as reduced female-typical sexual behaviors, upon adult estrogen treatment. The non-aromatizable androgen, 5alpha-dihydrotestosterone, produced very similar masculinizing and defeminizing effects. Likewise, in humans, androgens, and not estrogens, appear to be the principal masculinizing agents. This assumption has been primarily based on clinical reports of male individuals with either dysfunctional aromatase[13] or the alpha estradiol receptor,[14] as well as some of the DSDs in which androgen levels or actions have been altered (e.g., CAH and CAIS). It should be noted, however, that recent genetic studies[15] analyzing polymorphisms in genes encoding steroid hormone receptors and aromatase in relation to variants in gender identity have suggested a particular role for the beta estradiol receptor interacting with the androgen receptor in brain masculinization.

CAH is one of the most common DSDs (1:10,000) and has shown to be an interesting "model" for analyzing the role of prenatal androgens in the sexual differentiation of the brain. In 95% of CAH cases, a mutation in the gene encoding the enzyme 21-hydroxylase, which is important for the conversion of progesterone to deoxycorticosterone, has been described. As a consequence, progesterone is converted into 17-hydroxyprogesterone and ultimately into androgens. The absence of any cortisol production by the adrenal glands affects the general functioning of the hypothalamic-pituitary-adrenal axis, such that there are no longer any negative-feedback actions of cortisol on the release of either hypothalamic corticotropin-releasing hormone or pituitary adrenocorticotropic hormone, leading to an overproduction of adrenal androgens. This mutation affects both males and females, although the most profound effects are

observed in girls. They are often born with ambiguous genitalia depending on the degree of androgen exposure during fetal development. Therefore, they are generally diagnosed and treated shortly after birth, in particular to supplement them with glucocorticoids to restore cortisol levels and to reduce androgen levels. They are typically reared as girls.[16]

Thus, females with CAH have often been investigated to determine whether androgens affect the brain and behavior in humans as has been shown in primate species. If true, then females with CAH should be more male-typical compared to females without CAH. In particular, numerous studies have focused on sex-typed behaviors such as toy and activity preferences in girls with CAH because large and robust sex differences have been described in these behaviors and activities.[17] It was repeatedly found that, starting early in childhood, girls with CAH typically show masculinized patterns of sex-typed behaviors such as toy and activity preferences (i.e., spending more time playing with boys' toys such as cars than girls' toys such as dolls, showing more rough and tumble play, and preferring boys over girls as playmates).[18-20] It was further found that CAH girls were less responsive than non-CAH girls to information that particular objects are for girls, and they also mimicked the choices of other girls less when choosing particular objects.[21] These latter findings suggest that prenatal androgen exposure may affect later gender-related behaviors, including object (toy) preferences, in part by changing processes involved in the self-socialization of gendered behavior. By contrast, spatial abilities were not masculinized in CAH females.[22] It has generally been assumed that sex differences in spatial abilities, which generally favor males, reflect early androgen exposure. Likewise, less robust results have been found regarding sexual orientation and gender identity. Although there is an increased incidence of bisexual orientation and gender incongruence,[23] the vast majority of CAH women are heterosexual and identify themselves as women. Taken together, studies in CAH show that not all behavioral domains are equally affected by early androgen exposure.

Androgen insensitivity syndrome (AIS) in genetic 46 XY males is another DSD that has been shown to be useful for investigating organizational actions of androgens on the brain. In addition, this DSD is a very interesting "model" for determining direct sex chromosome effects on the brain. AIS is characterized by mutation(s) in the androgen receptor gene,[24] which lies on the X chromosome. It has an estimated incidence of 1:4000 to 1:99,000[25] depending on the form, from mild (MAIS), partial (PAIS) to complete (CAIS) androgen resistance. MAIS is characterized by a male phenotype with fertility problems, whereas mild to severe hypomasculinization with ambiguous genitalia can be observed in PAIS. CAIS, on the other hand, have completely abolished androgen receptor function and present a female phenotype. Thus, in a fetus with CAIS, the presence of the SRY gene on the Y chromosome induces the development of testes that not only produce testosterone, but also anti-müllerian hormone (AMH). Because they do not have any functional androgen receptors, there are no typical male internal and external genitalia, and instead typical female external genitalia develop. Furthermore, AMH induces regression of the Müllerian ducts, resulting in neither male, nor female typical internal genitalia (no uterus and blind-ended vagina). At birth, they are typically assigned as female. CAIS is either detected in infancy in the case of an inguinal hernia or later in adolescence when presenting primary amenorrhea.[25] Interestingly, they show spontaneous breast development. It has been postulated that CAIS might be more sensitive to estrogens (derived from aromatization of testosterone).[26] The testes are often surgically removed because there is an increased risk of testicular tumor development.[27,28] Estrogen replacement therapy is then usually initiated to induce puberty in case of gonadectomy before puberty, as well as to optimize bone health later on.[29] Most CAIS report an androphilic sexual orientation (i.e., sexual attraction to men), a female gender identity and a female-typical gender role,[30-32] supporting the notion that, in the absence of androgens, typical female psychosexual characteristics develop. However, a small percentage of CAIS do not report a female gender role and/or identity or an exclusively androphilic sexual orientation.[33,34] Gender development might thus not always be typically female in CAIS, suggesting a potential contribution of the sex chromosome genes as well.

SCAs represent an additional opportunity for investigating the contribution of sex chromosome genes to the sexual differentiation of the brain. SCAs are characterized by having an atypical number of sex chromosomes. The most studied SCAs are KS (incidence, 1:500 to 1:1000) characterized by two or more X chromosomes and a Y chromosome, thus male phenotype (testes), and TS (incidence 1: 2500), characterized by one X chromosome and a lack of (all or part) of the second X chromosome, and thus female phenotype (ovaries). However, SCAs have some limitations, such as that they are not euploid by definition and so any observed effects of sex chromosome dosage might not be applicable compared to XX females and XY males. Furthermore, regarding parent of origin, in XY males, the X is always inherited from the mother, which is not necessarily the case in SCAs. In addition, some SCAs and specifically KS and TS also result in atypical steroid hormone levels such as hypogonadism in KS men[35] and decreased estradiol production in TS women as a result of premature ovarian failure.[36] Therefore, it has been challenging to determine whether any effects observed in KS and TS are actually the result of sex chromosome dosage and/or steroid hormone effects. Nevertheless, studies on SCAs have shown that sex chromosome dosage affect overall brain size and, in particular, supernumerary X-chromosomes are associated with reductions in total brain volume in both males and females.[37]

4 | NEUROIMAGING STUDIES

The introduction of neuroimaging techniques such as magnetic resonance imaging (MRI) and positron emission topography has made it possible to investigate brain structure and function in CAH and CAIS populations and thus the analysis of organizational effects of prenatal androgens and/or sex chromosome genes.

4.1 | Prenatal androgen effects on brain structure

Many neuroimaging studies have focused on sex differences in overall or regional gray (GM) volumes derived from structural MRI scans (for meta-analysis[38]). Overall sex differences have been found in total brain size, with the male brain being on average 11% larger than the female brain. At the regional level, robust sex differences have been found in the amygdala, hippocampus, insula and parts of the frontal cortex, among many other regions.

Most neuroimaging studies on CAH have shown several structural alterations, such as reduced whole brain volume and altered prefrontal, parietal and superior occipital cortices.[39] Because these alterations have been observed in both men and women with CAH, they are most likely not specifically related to prenatal androgen exposure, but rather to their glucocorticoid treatment or other disease-related aspects, such as an overall decrease in working memory performance.[40,41]

Neuroimaging studies on CAIS have been more informative. In a recent study,[42] cortical thickness and subcortical GM volumes were compared between 16 women with CAIS and groups of control men and women ($n = 32$ per sex). A female typical pattern was found in CAIS in some brain regions (i.e., similar to control women, they had thicker parietal and occipital cortices and a thinner left temporal cortex, and larger volumes of the hippocampus than control men). These findings suggest that these sex differences are established under the influence of androgens. However, CAIS women also showed a male pattern in some brain regions, such as a significantly thinner cortex in the precentral and postcentral gyrus and smaller volume of the caudate nucleus, compared to control women, and thus were similar to control men. Furthermore, CAIS had larger overall brain volumes compared to control women, which might be related to their height because they are generally taller than control women. Taken together, CAIS women showed a mixed male and female pattern in brain structure, suggesting direct effects of sex chromosome genes in addition to steroid hormone effects.[42]

Using diffusion tensor imaging, a MRI technique to analyze white matter (WM) characteristics, important sex differences are observed in major WM regions.[43-46] These sex differences were replicated in a study including CAIS women.[47] Interestingly, CAIS women showed female-typical WM characteristics throughout the WM skeleton and did not differ from control women in any of these measures (but strongly from control men). These findings suggest a more important role for steroid hormones than for sex chromosome genes in the sexual differentiation of WM microstructure.

4.2 | Prenatal androgen effects on brain function

Neuroimaging studies on brain function in CAH have primarily focused on amygdala function in response to emotional pictures and predominantly in adolescents. In one such study,[48] it was found that girls with CAH showed a similar pattern of activation of the amygdala when viewing negative faces as control boys (i.e., increased activation of the amygdala). By contrast, CAH boys did not differ from control boys. These results thus suggest a potential role of prenatal androgens in emotional processing by the amygdala.

Neuroimaging studies in CAIS have focused on brain responses to sexually arousing stimuli[49] or spatial abilities[50] because robust sex differences have been observed in these two domains.[51,52] In the study by Hamann et al.,[49] men showed greater activation in the amygdala when viewing sexual images compared to control women and women with CAIS, with the latter two groups not being different. In the study by Van Hemmen et al.,[50] control men responded faster than women with CAIS when performing a 3D mental rotation task, although no differences were observed in accuracy scores between the groups. At the neural level, the overall pattern of activation was consistent with results from a meta-analysis on neuroimaging studies during mental rotation.[53] Sex differences were found with control men showing significantly more activation than control women in the left inferior parietal lobe. Neural activation in CAIS women differed significantly from control men and resembled that of control women. These results support the notion that sex differences in brain activation when performing a spatial ability task most likely reflect androgen exposure and is not directly driven by genetic sex.

Functional MRI (fMRI) studies of the human brain have suggested that low-frequency fluctuations in resting state fMRI data collected using blood oxygen level dependent (BOLD) contrast-based MRI correspond to functionally relevant resting state networks.[54] In the study by Savic et al.,[42] overall, women with CAIS showed functional connectivity similar to that of control women. For example, both control women and women with CAIS showed stronger functional connections in the posterior cingulate and precuneus, part of the default mode network (DMN), compared to control men. Likewise, the connections from the left amygdala to the anterior and posterior cingulate, as well as between the right and left amygdala, were stronger in CAIS and control women than in control men. By contrast, control men showed stronger right and left amygdala connections to the motor and insular cortices compared to CAIS and control women. The sex differences observed in the DMN with a stronger functional connectivity in women than in men probably reflect fetal actions of testosterone. In a recent study,[55] higher levels of fetal testosterone measured in amniotic fluid were associated with a reduction of functional connectivity in the DMN of adolescent males, but not of females.

Some caution is warranted in the interpretation of the results obtained in CAIS. First, all women with CAIS identified themselves as women and were reared as girls[49,50] (data on gender identity/role not provided in Savic et al.[42]). Exposure to typically masculine toys and activities is assumed to enhance performance on spatial tasks.[56] Because recalled childhood toy and activities preferences were sex typical in the study by Van Hemmen et al.[50] (i.e., there was a greater preference for masculine toys and activities in control men and for feminine toys and activities in control women and women with CAIS), it cannot be ruled out that their performance and neural activation reflects female-typical socialization.

Furthermore, female typical neural activation patterns observed in CAIS may also reflect estradiol exposure because they have supposedly higher circulating estrogen levels than men as a result of aromatization of testosterone to estradiol when the gonads are still *in situ*[57] or as a result of estrogen replacement therapy following gonadectomy. Some neuroimaging studies have indeed observed that mental rotation-related performance[58] and neural activation[59] fluctuated as function of the menstrual cycle and thus presumably as a function of circulating estradiol levels, although other studies have not reported such effects of estradiol.[60,61]

It is also possible that estradiol derived from testosterone aromatization has been organizing the CAIS brain in a female direction because the aromatase enzyme is already present early in development.[62] Recent studies[6,63] in mice have shown that estradiol is actually required during a specific prepubertal period for the development of the neural circuit mediating female sexual behavior. Kisspeptin neurons in the anteroventral periventricular area (AVPV) are a potential target of estradiol action in the female mouse brain because this particular neuronal population develops under the influence of prepubertal estradiol.[64,65] In addition, it was recently shown that AVPV kisspeptin neurons are crucial for the expression of female sexual behavior in mice and actually represent a central hub in the neural network orchestrating sexual behavior with ovulation in female mice.[66] These results thus challenge the classical view of a default organization of the female brain. To date, in humans, very little is known about whether there is an active, estradiol-induced, feminization of the brain. However, some hints are derived from studies[67-69] reporting that aspects of heterosexual function were significantly lower in TS women. Postmortem studies[70,71] have shown that, in humans, similar sex differences exist in the number of kisspeptin neurons (with women showing greater numbers than men) at various periods of life (infant/prepubertal, adult, elderly) in the infundibular nucleus, although these studies have unfortunately not revealed whether the female typical population develops under the influence of estradiol. Furthermore, whether kisspeptin plays a similar role in female sexuality, as has been observed in mice, remains to be determined. Clearly, more research is needed to elucidate the role of estrogens in the development of the brain, as well as whether they affect brain structure and function in adulthood.

5 | ROLE OF PUBERTAL HORMONES IN NEURODEVELOPMENT: DATA DERIVED FROM ADOLESCENTS WITH GENDER INCONGRUENCE

Gender incongruence (ICD-11), also known as gender dysphoria (DSM-5), is defined as a marked and persistent incongruence between an individual's experienced gender and the at birth assigned sex. Feelings of gender incongruence can already be present in early childhood since gender identity (i.e., someone's fundamental sense of self as being male or female) develops early in life. Indeed, children start to use gender labels in their speech at between 1 and 2 years of age and, by the age of 2–3 years, they often show gender-typical play behavior and a preference to play with gender stereotyped toys.[72] At those early ages, children also are able to refer to themselves as a boy or a girl.[72] Generally, gender identity is further consolidated during adolescence, a period of significant cognitive and social-emotional changes, when adolescents start to explore their sexual identity.[73] In the case of gender incongruence, it has been suggested that early adolescence (between 10 and 13 years of age) forms a critical developmental period in the (dis-)continuation of gender dysphoric feelings. The few longitudinal studies that have allowed estimates on the development of gender incongruence demonstrated that only part of the childhood cases (39%–40%)[74,75] will show persisting gender dysphoria and start puberty suppression treatment at adolescent age.

A prominent hypothesis on the etiology of gender incongruence proposes that the condition is related to the sexual differentiation of the brain and specifically to the fact that different critical periods exist for the development of the reproductive organs vs. the brain, thereby proposing that these two processes might have been affected differentially in individuals with gender incongruence.[76-79] This hypothesis is mostly based on postmortem studies investigating the brains of individuals with gender incongruence. For example, a female-typical volume and number of neurons in the central subdivision of the bed nucleus of the stria terminalis and the third interstitial nucleus on the anterior hypothalamus have been observed in transgender females (male sex assigned at birth and female gender identity).[80-82] In addition, a female-typical expression of neurokinin B and kisspeptin, two neuropeptides important in the regulation of gonadotropin-releasing hormone (GnRH) neuronal activity, and thus reproductive functioning, has been detected in the infundibular nucleus of the hypothalamus of transgender females.[71,83] These postmortem studies have provided valuable information on the neurobiological underpinnings of gender incongruence. However, caution is warranted when interpreting the results because of the relatively small sample size, as well as the potential influence of hormonal therapy that most transgender females received at some point during their life. Thus female-typical hypothalamic volumes might also be the result of, rather than, the cause of life-long gender incongruence and/or cross sex hormone use.

The introduction of neuroimaging techniques has made it possible to conduct larger-scale studies involving more substantial subject samples and a more controlled study design (i.e., to account for hormonal treatment as well as sexual orientation). Since 2010, the number of both structural and functional neuroimaging studies on the brain in individuals with gender incongruence has been steadily increasing, although still remains rather low in comparison with other topics covered in neuroscience. Because it is not the scope of this review to provide a detailed overview of all neuroimaging studies conducted in relation to gender incongruence, further reading is available elsewhere.[84,85]

Generally, gender affirming treatment has been met with a great deal of skepticism, which is even more pronounced when it comes to the treatment of minors. However, this policy has changed over

the years, albeit this can be strongly dependent on the country. In the Netherlands, Cohen-Kettenis and Delemarre-van de Waal have been pioneers in terms of establishing a treatment protocol for minors: early adolescents experiencing severe gender incongruence, are allowed, upon a careful psychological diagnostic procedure, to start using GnRH agonists (GnRHa) to suppress pubertal maturation.[86-89] The advantages of this treatment are that (1) any further development of the secondary sex characteristics is halted, (2) psychological well-being improves (stress relief), (3) they can take time to weigh the possibilities of a sex reassignment procedure and (4) halting the development of the secondary sex characteristics positively influences later physical appearance. Nevertheless, this treatment protocol still receives some criticism, particularly with regard to arresting pubertal development. It has been argued that experiencing all stages of puberty and having age-appropriate sociosexual experiences is crucial for psychological maturation, both at the cognitive and emotional level.[90-92] It is well-known that the prefrontal cortex, a brain area critical for cognitive control, emotion regulation and social cognition, shows protracted development during adolescence.[93,94] It can thus be argued that inhibiting pubertal development might interfere with the marked adolescent-specific developmental changes in cognition, and, consequently, their ability to reflect upon something important as sex reassignment. Finally, several studies conducted in animal models[6,95] have suggested that puberty might reflect a second, organizational period in brain development and thus exposure to pubertal hormones is necessary for full neural development.

The current literature on puberty and brain development in humans is still rather limited and has not specifically addressed any differential effects of pubertal timing and thus whether delaying puberty might potentially affect brain development. There is evidence that developmental trajectories of certain brain areas align better with pubertal changes and, in particular, hormone levels than with age,[96-98] although age-related effects have not been specifically investigated. At present, there appears to be only one study[99] that has explicitly tested the relationship between pubertal timing and sex-typical cognitive development. It was found that mental rotation ability in men was inversely associated with recalled timing of puberty. Taken together, these studies suggest that pubertal hormones may have organizational effects on cognitive functioning, and there might also be a decreasing window of sensitivity to testosterone throughout adolescence. The latter could be highly relevant in light of the timing of treatment of transgender youth because they have to wait until the age of 16 years before being treated with cross-sex hormones and thus undergo full puberty. Thus, treatment with GnRHa might produce a myriad of varied impacts, both positive and disruptive.

At present, only very few studies[100,101] have assessed the impact of pubertal suppression on neural and cognitive functioning in transgender youth. Staphorsius et al.[100] analyzed the effects of pubertal suppression on executive functioning by measuring brain activity using fMRI when performing the Tower of London task. In this task, two boards with pegs and several beads with different colors are presented simultaneously. One board represents the start configuration and the other board the target (goal) configuration. Participants are asked to preplan mentally the minimum number of steps (ranging from 1 to 5) required to reach the target configuration. This particular task has been shown to improve with age until early adulthood and thus to correlate nicely with the development of the prefrontal cortex.[102-105] Furthermore, reduced performance has been observed on this task in patients with pathology of the prefrontal cortex.[106] Thus, patterns of neural activation of adolescents with gender incongruence who received GnRHa to suppress puberty were compared with those of cisgender adolescents. In addition to determining whether potential differences between the groups were a result of GnRHa treatment and not associated with the diagnosis of gender incongruence, a group of age-matched adolescents with gender incongruence who were not yet using GnRHa but were already in puberty, was included in the study. It should be noted that, in this particular study,[100] participants were matched by age and not by Tanner stage. All GnRHa treated adolescents had reached at least Tanner stage 2 before being allowed to start puberty-suppressing treatment. However, the Tanner stages of the other groups were not recorded. In later adolescent MRI studies, Tanner stages have been used[107,108] because they are more informative about the level of hormonal exposure experienced.

At the behavioral level, it was found that transgender girls (male sex assigned at birth, female gender identity) receiving GnRHa had significantly lower accuracy scores than the cisgender groups and untreated transgender boys (female sex assigned at birth, male gender identity). They also had the lowest IQ scores and, even though there was a significant correlation between accuracy on the Tower of London task and IQ, a significant effect of group on accuracy remained even after correcting for IQ. Possibly this might be a chance finding because this group was rather small ($n = 8$). Other groups did not differ and no sex differences were observed.[100]

At the neural level,[100] region of interest analyses showed sex differences, with cisgender boys showing significantly greater activation than cisgender girls during high task load Tower of London items in the bilateral precuneus and a trend for greater activation in the dorsolateral prefrontal cortex. By contrast, brain activation levels of untreated transgender adolescents were intermediate between those of the two cisgender control groups in those brain areas that showed significant sex differences. Thus, untreated transgender girls and boys had a closer resemblance to each other than the cisgender groups and no sex differences were found. Similar results have been found in another study in which verbal fluency was analyzed.[109] In this particular study, the cisgender groups showed a sex difference in the activation of the right rolandic operculum, a small area adjacent to Broca's area, although the untreated transgender adolescents showed intermediate activation and no sex difference. As proposed by the sexual differentiation hypothesis of gender incongruence,[110-112] the absence of a sex difference in untreated transgenders might be a result of a different hormonal milieu during prenatal development. However,

possible effects of pubertal hormones on establishing sex-atypical activation patterns cannot be ruled out based on the results of the untreated participants.

Interestingly, puberty-suppressed transgender girls showed greater activation than the puberty-suppressed transgender boys not only in the same regions that were more active in cisgender boys than cisgender girls (i.e., the bilateral precuneus), but also the prefrontal cortex (left dorsolateral and bilateral rostrolateral), indicating even more pronounced sex differences, and thus sex-typical neural activations.[100] These results suggest that there are no obvious detrimental effects of GnRHa treatment on executive functioning. In addition, no evidence was found that GnRHa treatment would actually push transgender adolescents in the direction of their experienced gender. If anything, it was found that puberty suppression led to brain activation patterns that were more in accordance with their natal sex (in contrast to the neural activation patterns observed in untreated transgender adolescents).

In the clinical report by Schneider et al.[101] , a single pubertal transgender girl undergoing GnRHa treatment was evaluated at different time points. MRI scans were taken at baseline before starting GnRHa treatment and then at 22 and 28 months of pubertal suppression treatment. It was found that WM fractional anisotropy values did not increase in the manner normally expected during puberty. Furthermore, by 22 months of GnRHa treatment, working memory scores dropped by more than half a standard deviation, and then stayed stable at 28 months.

In sum, the study by Staphorsius et al.[100] did not show any obvious detrimental effects of GnRHa treatment (duration treatment 1.6 ± 1.0 years) in transgender youth, although the sample size was rather small and the treatment durations still rather short. The study by Schneider et al.[101] showed some disruptive effects of GnRHa treatment, although this was in only one transgender girl. Clearly, larger-scale, longitudinal studies are required to understand possible neurodevelopmental impacts of pubertal suppression over time in transgender youth.[113]

Interestingly, the same adolescent MRI sample[100] was used to analyze GM volumes.[114] At the whole-brain level, sex-typical volumes were observed in transgender youth. Some subtle deviations in GM volume in the direction of individuals sharing their gender identity were detected in transgender girls and boys only when specifically examining structures showing sex differences in the cisgender groups using region of interest analyses. This suggests only a partial sex-atypical differentiation of the brain in adolescents diagnosed with gender incongruence. These mixed results are in line with MRI studies conducted in adult transgender populations,[84,85] suggesting that there are most likely additional neural mechanisms underlying gender incongruence. Indeed, a second hypothesis based on functional MRI connectivity data proposes that gender incongruence could reflect a disconnection of fronto-parietal networks implicated in own-body self-referential processing.[115-117] However, it turns out that these two hypotheses are not contradictory, and instead are complementary. A recent study[118] in which global and regional connectivity differences within functional networks in transwomen and transmen with early-in-life onset gender incongruence were analyzed using three different methodologies confirmed the predictions from both hypotheses. The data showed significantly less frontal-parietal connectivity strength in transmen as predicted by the own-body perception hypothesis, although, on the other hand, cisgender men differed in connectivity strengths from cisgender women and both transgender groups (which did not differ among them), as suggested by the sexual differentiation hypothesis. Taken together, gender identity probably reflects an interaction between several brain networks controlling congruency between the sex assigned at birth and the feeling of being male or female.

6 | GENERAL CONCLUDING REMARKS

At present, strong evidence exists for a predominant role of steroid hormones in the sexual differentiation of the human brain. Data obtained from both CAH and CAIS point to an important role of (prenatal) androgens in inducing typical male neural and psychosexual characteristics in humans. Whether estrogens play a similar masculinizing role in humans as has been shown in rodent species, appears to be less clear, at least when studying DSDs and focusing on those domains showing strong sex differences (e.g., toy preferences, mental rotation). However, studies[15] in which steroid hormone receptor polymorphisms were investigated in relation to gender incongruence have suggested a specific role for the beta estradiol receptor in some aspects of brain masculinization. Specific allele combinations for steroid receptors were identified for transgenders (i.e., an inverse allele interaction between beta estradiol receptor and andogen receptor is characteristic of gender incongruence in natal males, whereas both estradiol receptors are associated with gender incongruence in natal females). However, at present, the mechanisms of how such steroid receptor polymorphisms will potentially lead to altered steroid hormone actions during brain development and, consequently, to gender incongruence, remain unknown.

In addition to a potential role in brain masculinization, estrogens might be involved in active brain feminization as has been suggested by some rodent studies, although the current evidence is rather limited to some observations made in TS women. It is difficult to address this particular question of potential estrogen actions in female neural development. It might be feasible by investigating psychosexual functioning in DSDs characterized by low to absent estradiol synthesis and secretion, such as Kallmann syndrome or any other GnRH-related deficiencies. However, these DSDs are very rare and also it might be difficult to exclude effects of gender socialization on any of these measures.

Interestingly, data from CAIS and SCAs also suggest a contribution of the sex chromosomes to the development of the brain, in particular with regard to brain structure. Thus, sex differences in the human brain appear to reflect a combination of steroid hormones, genetic factors and, last but not least, socialization-related effects. The relative contribution of each factor might depend on the brain area and/or function. This has been noticeable from studies in CAH

girls/women showing that not all behavioral domains are equally affected by early androgen exposure.

In sum, more research is clearly needed to better identify the different players in the sexual differentiation of the human brain. However, this will remain a rather challenging enterprise because such studies will depend primarily on natural occurring variations or differences in sex development and/or gender identity and, consequently, it will remain difficult to obtain sufficiently large sample sizes.

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CONFLICT OF INTERESTS

The author declares that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

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