The Sexual Differentiation of the Human Brain: Role of Sex Hormones Versus Sex Chromosomes

Julie Bakker

Contents

Abstract Men and women differ, not only in their anatomy but also in their behavior. Research using animal models has convincingly shown that sex differences in the brain and behavior are induced by sex hormones during a specific, hormone-sensitive period during early development. Thus, male-typical psychosexual characteristics seem to develop under the influence of testosterone, mostly acting during early development. By contrast, female-typical psychosexual characteristics may actually be organized under the influence of estradiol during a specific prepubertal period. The sexual differentiation of the human brain also seems to proceed predominantly under the influence of sex hormones. Recent studies using magnetic resonance imaging have shown that several sexually differentiated aspects of brain structure and function are female-typical in women with complete androgen insensitivity syndrome (CAIS), who have a 46 XY karyotype but a female phenotype due to complete androgen resistance, suggesting that these sex differences most likely reflect androgen action, although feminizing effects of estrogens or female-typical socialization cannot be ruled out. By contrast, some male-typical neural characteristics were also observed in women with CAIS suggesting direct effects of sex chromosome genes in the sexual differentiation of the human brain.

J. Bakker (✉)
Laboratory of Neuroendocrinology, GIGA Neurosciences, Liège University, Liège, Belgium
e-mail: jbakker@uliege.be

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In conclusion, the sexual differentiation of the human brain is most likely a multifactorial process including both sex hormone and sex chromosome effects, acting in parallel or in combination.

Keywords Androgens · Brain function · Brain structure · Complete androgen insensitivity syndrome · Estrogens · Magnetic resonance imaging · Sex differences · Sexual development

1 Introduction

Although men and women might be more similar than different, notable sex differences exist in cognitive abilities (Maccoby and Jacklin 1974), brain morphology (Cosgrove et al. 2007), emotion processing (Schirmer et al. 2004), and vulnerability to psychiatric disorders (Bao and Swaab 2011). The origin of these sex differences in the brain and behavior remains the subject of heated debate. In particular, whether sex differences observed in adulthood result from specific biological processes occurring during early development ("nature") or from the (social) environment ("nurture") has been contested. There is increasing evidence, however, that the organization of the brain is affected by circulating sex hormones and the expression of specific genes during early development and thus that sex differences in brain structure and organization are already present as early as birth. The biological bases of sex differences in brain and behavior are becoming better known, but many questions remain about how sex hormones induce such different patterns of neural and behavioral differentiation in men and women. This chapter will provide a short overview of our current knowledge of how the sexual differentiation of the human brain proceeds under the influence of organizing actions of sex hormones. For obvious ethical reasons, many of our ideas about the sexual differentiation of the human brain are derived from (clinical) research on patients with disorders of sex development (DSD).

1.1 Sex Determination and Sexual Differentiation

In mammals, including humans, the developing organism has the potential to become either male or female. The first step in the process of sex determination is the establishment of the genetic sex at conception. Each cell nucleus contains a species-specific number of paired autosomes and two sex chromosomes. Humans have 22 pairs of autosomes and one pair of sex chromosomes (the X and Y). Autosomes are numbered roughly in relation to their sizes, i.e., chromosome 1 has approximately 2,800 genes, whereas chromosome 22 has approximately 750 genes. The sex chromosomes will determine the sex of the fetus: females have two X chromosomes, whereas males have one X and Y chromosome. The latter contains

the *Sry* gene, which will induce the formation of testes from the undifferentiated gonads in males (Koopman et al. 1990). The Leydig cells in the testes will produce testosterone which will promote the development of the Wolffian ducts into the internal male genital structures such as the epididymis, the vas deferens, and the seminal vesicles, whereas anti-Müllerian hormone secreted by the Sertoli cells in the testes causes regression of the female-typical Müllerian ducts. The penis and scrotum develop under the influence of dihydrotestosterone which is formed from testosterone by the enzyme, 5α-reductase. In typical female differentiation, i.e., in the absence of the Y chromosome and consequently the *Sry* gene, the undifferentiated gonads will develop into ovaries. The Müllerian ducts develop without any apparent hormonal input into the uterus, fallopian tubes, and the distal portion of the vagina, whereas the Wolffian ducts regress and disappear in the absence of androgenic stimulation.

1.2 Sexual Differentiation of the Brain: Animal Studies

Animal studies have convincingly shown that the sexual differentiation of the brain generally follows the same pattern as that of the genitals, i.e., exposure to testosterone induces male-typical psychosexual and neural characteristics, whereas in the absence of testosterone, female-typical psychosexual and neural characteristics develop, i.e., by default. This is mainly based on studies in rodents in which the testes were removed directly after birth or conversely, when testosterone was administered to newborn females. It was found that neonatally castrated male rats showed very few male-typical sexual behaviors even following administration of testosterone in adulthood (Grady et al. 1965), whereas testosterone-treated females showed increased levels of male-typical sexual behaviors, such as mounting behavior (reviewed in Baum 1979). In addition, when treated with "female-typical" sex hormones, i.e., estradiol and progesterone, neonatally castrated male rats showed female-typical sexual behaviors, like the expression of the typical female rodent mating posture, lordosis (Feder and Whalen 1964). This led to the conclusion that the female-typical differentiation of the brain proceeds in the absence of any hormonal secretion, i.e., by default. This was further supported by the finding that the ovaries are functionally quiescent during early development: no significant amounts of estradiol could be detected before postnatal day 7 (Lamprecht et al. 1976), whereas the testes start to produce testosterone about 1 week before birth in rodents. However, more recent studies have shown that estradiol may be required for the development of the female brain. Female aromatase knockout (ArKO) mice which carry a targeted mutation in the aromatase gene and as a result cannot synthesize estrogens from androgens, show reduced levels of female sexual behavior in adulthood, even following ovariectomy and subsequent treatment with estradiol and progesterone (Bakker et al. 2002). Interestingly, administration of estradiol over a specific prepubertal period (postnatal days P15–P25) almost completely restored female sexual behavior in

female ArKO mice (Brock et al. 2011). This result clearly challenges the classical theory of a default organization of the female brain. It also challenges the idea that sex differences are established before or directly after birth and that sex hormones beyond the perinatal period only have so-called "activational" effects on the brain. Hence, it might be well possible that the brain remains plastic and sensitive to any organizational actions of sex hormones for a much longer period as initially thought.

Although very little doubt remains on the pivotal role of gonadal hormones in establishing sex differences in the brain and behavior, some evidence has been emerging that genes on the sex chromosomes might also contribute to the sexual differentiation of the brain (Arnold et al. 2004). By using a core-cross transgenic mouse model in which the *Sry* gene was deleted from the Y chromosome and inserted into an autosome to create XX and XY male and female phenotypes, it was possible to distinguish between the contribution of gonadal hormones and sex chromosome genes to the development of sex differences in the brain and behavior. It was shown, for instance, that the male-typical profile of vasopressin innervation of the lateral septum depends on the presence of a Y chromosome (de Vries et al. 2002). XY males and XY female mice (i.e., females with a deletion of the *Sry* gene) were more masculine than XX mice in the density of vasopressin-immunoreactive fibers in the lateral septum. Based on these findings, it has been proposed that the mechanism of sexual differentiation is multifactorial and includes both sex hormone and sex chromosome effects, acting in parallel or in combination (Arnold 2017).

1.3 Sexual Differentiation of the Human Brain

An important question raised by animal studies is that if sex hormones play such a pivotal role in masculinizing or feminizing the brains of nonhuman species, do they have similar actions in our own species? In other words, do men and women behave differently because men have been exposed to higher concentrations of testosterone during development or conversely, women to higher levels of estrogens? In humans the sexual differentiation of brain is thought to occur between 8 and 24 weeks of gestation when testosterone levels are higher in male than female fetuses (Reyes et al. 1974; Nagamani et al. 1979). Furthermore, the first months after birth are also marked by a testosterone surge 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 (Winter et al. 1976; Kuiri-Hänninen et al. 2014). This period is often referred to as "mini-puberty" (Kuiri-Hänninen et al. 2014), is most likely caused by an increased gonadotropin secretion since there is no longer any negative feedback by estrogens after birth, and might be an additional critical period for organizational actions of sex hormones on the brain and behavior.

1.4 Indirect Measures of Prenatal Hormone Levels

For obvious ethical reasons, indirect markers of prenatal hormone levels have been used to study potential organizational effects of sex hormones in the human brain. The 2D:4D ratio, i.e., the relative length of the second to the fourth digit, which is larger in women than men (Hönekopp and Watson 2010), is the most extensively used marker (Morris et al. 2004), because it is so easy to obtain, a simple photocopy of the hand suffices. However, its validity as an indicator of prenatal androgen exposure has been criticized (Berenbaum et al. 2009; van Hemmen et al. 2017a). Firstly, two independent studies (Lutchmaya et al. 2004; Ventura et al. 2013) have actually attempted to measure fetal androgen levels through amniocentesis and to correlate them with digit ratios, but their results were inconclusive. Secondly, the results obtained in women with complete androgen insensitivity syndrome (CAIS) strongly suggest that other non-androgenic factors are also involved (van Hemmen et al. 2017a). Otoacoustic emissions (OAE) are sounds produced by the cochlea, which can be measured in the inner ear canal (Kemp 1978, 2008; Davis 1983), and are another marker used for assessing prenatal hormone exposure retrospectively. Spontaneous OAEs are more frequent and stronger in women than in men. Likewise, OAEs evoked by click stimuli have larger amplitudes in women than in men (McFadden and Pasanen 1998; Shinur and Hampson 2011). These two measures have in particularly been used to assess whether sexual orientation might reflect changes in prenatal androgen exposure (for comprehensive review, see Breedlove 2017). It was found, however, that homosexual men showed very similar 2D:4D ratios and OAEs as heterosexual men. By contrast, lesbian women showed "masculinized" 2D:4D ratios and OAEs suggesting that they might have been exposed to increased levels of androgens during early development. Although these markers might be useful to determine early androgen exposure, they have many limitations since they can only be used retrospectively and might also be affected by circulating hormone levels in adulthood. This has particularly been suggested for OAEs (Shinur and Hampson 2011).

1.5 Postmortem Studies

Postmortem analyses of the brain have been an important method to determine whether sex differences in human behavior, but also differences related to sexual orientation and gender identity, could be explained by structural differences in the brain. Many of these studies have been inspired by observations made in animal studies. For instance, the discovery of a sexually dimorphic nucleus (SDN) in the rat preoptic area led to a close examination of the human preoptic area where a similar sexually dimorphic nucleus was observed (SDN or interstitial nucleus of the anterior hypothalamus (INAH-1 and 2)) which is larger in men than in women. However, no variations in the size of this nucleus have been observed in relation to sexual

orientation (Swaab 2007). In addition, three independent groups have shown that a different nucleus in the anterior hypothalamus, INAH-3, is significantly larger in heterosexual men than in heterosexual women (Allen et al. 1989; Byne et al. 2001; Levay 1991), and in a highly publicized study (LeVay 1991), it was reported that the volume of INAH-3 was greater in heterosexual than in homosexual men, although this latter finding still awaits full replication (Byne et al. 2001). A rather unexpected finding (Swaab and Hofman 1990) was that the suprachiasmatic nucleus (SCN), the clock of the brain, was twice as large in homosexual men compared to heterosexual men, in particularly because no sex differences were observed. This finding suggests that homosexual men do not have a "female-typical" hypothalamus as has been proposed (Dörner 1988).

By contrast, female-typical volumes of the central nucleus of the bed nucleus of the stria terminalis (Zhou et al. 1995; Kruijver et al. 2000) and the INAH-3 (Garcia-Falgueras and Swaab 2008) have been observed in male-to-female (MtF) transsexuals. Likewise, a female-typical expression of two neuropeptides important in regulating GnRH secretion, i.e., neurokinin B (Taziaux et al. 2012) and kisspeptin (Taziaux et al. 2016), has been found in the infundibular nucleus of the hypothalamus of MtF transsexuals. These results may suggest that transsexual people, which are now referred to as people diagnosed with gender dysphoria (defined as a strong incongruence between their gender assigned at birth and the gender that they identify with; DSM-5), have undergone an atypical sexual differentiation of the brain. However, some caution is warranted in interpreting the results because some of these effects might be due to adult hormone treatment (for instance, estrogen treatment in MtF transsexuals) or other not-yet-identified causes.

1.6 Clinical Studies

Perhaps more valuable insights into the mechanisms underlying the sexual differentiation of the human brain might be obtained by studying "disorders of sex development" also known as "disorders of sex differentiation" (DSD). The term DSD refers to congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical (as cited in Hughes et al. 2006). Although many DSDs are not well studied in this context because they are extremely rare, several DSDs provide a unique opportunity to assess the different players involved in sexual differentiation. Examples include, but are not limited to, congenital adrenal hyperplasia (CAH), sex chromosome aneuploidies, and complete androgen insensitivity syndrome (CAIS).

Congenital adrenal hyperplasia (CAH) is the most common DSD (1:10,000) which is characterized in about 95% of the cases by a mutation in the gene encoding the enzyme 21-hydroxylase which is important for the conversion of progesterone to deoxycorticosterone. As a result, progesterone can only be converted to 17-α-hydroxyprogesterone (which in turn cannot be converted to 11-deoxycortisol). The lack of cortisol will lead to an increased release of adrenocorticotropic hormone

(ACTH) by the anterior pituitary since cortisol normally has a negative feedback action on ACTH as well as on the secretion of its stimulator corticotropin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus. This increased stimulation by ACTH induces overgrowth (hyperplasia) and hyperactivity of the steroid-producing cells of the adrenal cortex ultimately leading to an increased production of androgens during fetal life. At birth, 46, XX females often have ambiguous genitalia depending on the degree of exposure to androgens during fetal development. Starting early in childhood, girls with CAH also typically show masculinized patterns of sex-typed behavior such as toy and activity preferences, i.e., spending more time playing with boy's toys such as cars than girls' toys such as dolls, showing more rough and tumble play, and preferring boys over girls as playmates (Collaer and Hines 1995; Pasterski et al. 2005). In adulthood, CAH is often associated with polycystic ovarian syndrome (oligomenorrhea, polycystic ovaries, hirsutism) and increased incidence of bisexual orientation and gender dysphoria (Meyer-Bahlburg et al. 2008). In contrast, 46, XY boys with CAH do not show any clear symptoms other than those related to decreased cortisol production. These observations in girls/women with CAH suggest that prenatal androgens indeed might organize male-typical psychosexual and neurobehavioral characteristics.

Individuals with sex chromosome aneuploidies (SCAs) have an atypical number of sex chromosomes and often show deficits in certain cognitive domains (Printzlau et al. 2017), some of which showing sex differences in the general population. Therefore, SCAs have been suggested as a method to study possible effects of sex chromosome genes on the development of the brain. Klinefelter syndrome (KS; incidence, 1:500–1,000), characterized by two or more X chromosomes and a Y chromosome, thus male phenotype, and Turner syndrome (TS; incidence, 1:2,500), characterized by one X chromosome and a lack of (all or part) of the second X chromosome, thus female phenotype, have been mostly studied in this context. However most SCAs also result in atypical sex hormone levels, such as hypogonadism in men with KS prior to being supplemented with testosterone (Davis et al. 2015) and decreased estradiol production in women with TS due to premature ovarian failure (Modi et al. 2003). Consequently, it has been challenging to establish whether any findings obtained in these two SCAs represent direct genetic effects related to sex chromosome gene dosage, sex hormone effects, or both.

Androgen insensitivity syndrome (AIS) might be one of the most interesting DSDs to study the respective roles of sex hormones versus sex chromosome genes in the sexual differentiation of the human brain. AIS has an estimated incidence of 1:40,800 to 1:99,000 (Boehmer et al. 2001) and is characterized by a mild (MAIS), partial (PAIS), or complete (CAIS) defect in androgen action caused by mutation(s) in the X-chromosome-linked androgen receptor gene (Hughes et al. 2012), resulting in decreased or completely abolished AR function. The degree of androgen resistance determines the phenotypical presentation, ranging from a male phenotype with fertility problems in MAIS to mild or severe hypomasculinization and ambiguous genitalia in PAIS and a female phenotype in CAIS. In a fetus with CAIS, the gonads develop into testes under the influence of the SRY gene and start producing

androgens and AMH. Due to the inability of androgens to activate the AR, the external genitalia develop in the female direction, whereas AMH causes regression of the Müllerian duct, resulting in a blind-ending vagina and absent uterus. CAIS is detected in infancy in case of an inguinal hernia or in adolescence in case of primary amenorrhea (Hughes et al. 2012). The assigned gender at birth and gender upbringing is typically female. With regard to secondary sex characteristics, pubic and axillary hair is either sparse or absent (Tadokoro-Cuccaro and Hughes 2014) since they depend on androgens. There is spontaneous breast development since testosterone is converted into estradiol by the enzyme aromatase, and CAIS might be more sensitive to estrogens (Zachmann et al. 1986). Because of an increased risk of gonadal tumor development in DSDs, including CAIS, the general medical advice is to surgically remove the gonads (Cools et al. 2006; Lee et al. 2016). Following gonadectomy, estrogen replacement therapy is initiated to induce puberty in case of prepubertal gonadectomy and to optimize bone health later on (Bertelloni et al. 2011). Studies on the psychosexual development of CAIS have generally shown an androphilic sexual orientation (i.e., sexual attraction to men), a female gender identity, and female-typical gender role behavior (Masica et al. 1971; Wisniewski et al. 2000; Hines et al. 2003) which is in line with a hypothesized role of androgens in the sexual differentiation of these psychosexual characteristics. Recent work (T'Sjoen et al. 2011; Brunner et al. 2016), however, showed that some individuals with CAIS reported other-than-female gender roles or neither-female-nor-male genders. In addition, not all CAIS reported an exclusively androphilic sexual orientation. Gender development might thus not always be typically female in CAIS suggesting a potential contribution of the sex chromosome genes as well. However, for the rest of this chapter when discussing the results of the different neuroimaging studies, we will refer to women with CAIS because they all identified themselves as women in these studies and expressed a very strong desire to be called women.

1.7 The Sexual Differentiation of the Human Brain: Neuroimaging Studies in CAIS

1.7.1 Brain Function

The introduction of neuroimaging techniques such as magnetic resonance imaging (MRI) has made it possible to study sex differences in brain structure and function in the general population but also in clinical populations. Functional MRI (fMRI) studies, which measure the activity of the brain while performing a task, have, for instance, shown that neural activity while viewing sexual images, emotional stimuli, or during the performance of spatial tasks such as a three-dimensional mental rotation task (MRT) differs between men and women (reviewed in Sacher et al. 2013). Furthermore, MRI studies of brain structure have shown sex differences in the volume of and structural connectivity between brain regions (Gong et al. 2011; Ruigrok et al. 2014). Women with CAIS provide a unique opportunity to study the

origin of these sex differences and in particular to analyze the respective roles of sex chromosome genes versus androgens in the sexual differentiation of the human brain. At present, three independent groups (Hamann et al. 2014; van Hemmen et al. 2016, 2017b; Savic et al. 2017) have examined brain structure and function in CAIS using neuroimaging techniques. The fMRI study by Hamann et al. (2014) focused in particularly on brain responses to sexually arousing stimuli in light of the robust sex differences observed in this domain (e.g., Gizewski et al. 2009; Hamann et al. 2004). Men showed greater activation in the amygdala compared to control women and women with CAIS with the latter two groups not being any different. These results suggest that the male-typical activation most likely reflects androgen actions and thus no direct effects of genes of the Y chromosome. In addition, these data argue against an important role for brain aromatization in the masculinization of the brain as has been reported in some animal species (reviewed in Balthazart and Ball 2012) since women with CAIS have presumably higher brain estrogen levels derived from aromatization of testosterone but showed no signs of brain masculinization with regard to their responses to sexually arousing stimuli. However, all women with CAIS in this study (Hamann et al. 2014) identified themselves as women (gender identity and gender role) and were androphilic. They were also all raised as girls and received a female-typical socialization. Finally, it should be noted that group sizes were rather small ($n = 13$/group) and there was no information available on whether the women with CAIS were gonadectomized and/or received any hormone replacement therapy (HRT) nor a confirmation of their diagnosis other than self-report.

As mentioned previously, men and women differ in cognitive abilities with the mental rotation task, a visuospatial task, often showing the greatest sex differences, with men generally outperforming women (Linn and Petersen 1985). Accordingly, fMRI studies have demonstrated sex differences in neural activation while performing this task, with generally higher levels of activation in parietal regions in men (Jordan et al. 2002; Weiss et al. 2003; Butler et al. 2006; Gizewski et al. 2006; Schöning et al. 2007; Hoppe et al. 2012). By contrast, several studies showed a greater activation in frontal and temporal brain regions in women (Thomsen et al. 2000; Jordan et al. 2002; Weiss et al. 2003; Seurinck et al. 2004; Kucian et al. 2005; Butler et al. 2006; Gizewski et al. 2006; Schöning et al. 2007; Hoppe et al. 2012). Based on these findings from both behavioral and neuroimaging studies, it has been proposed that men and women use different strategies for solving this task: women are thought to use a serial and analytical approach, whereas men are thought to rely on a more automatic "gestalt" strategy (Thomsen et al. 2000; Jordan et al. 2002; Butler et al. 2006). It has been hypothesized that these sex differences most likely reflect early androgen actions since it has been shown that women diagnosed with CAH outperformed their unaffected sisters on spatial tasks in childhood, adolescence, and adulthood (Puts et al. 2008; Berenbaum et al. 2012). In addition, men who have had low androgen levels throughout life due to idiopathic hypogonadotropic hypogonadism showed impaired spatial abilities compared with control men (Hier and Crowley 1982). Androgen replacement therapy in these men did not provide any consistent results (Hier and Crowley 1982; Zitzmann et al. 2001), which could also

have been due to small sample sizes. The Wechsler Intelligence Scale for Adults (WAIS) and Wechsler Intelligence Scale for Children (WISC) have been used to study spatial abilities in women with CAIS. It was found that CAIS overall performed worse than control men and women (Imperato-McGinley et al. 1991; Masica et al. 1969). However, they were classified as being "feminine" based on their superior performance on verbal compared to spatial ability subtests. These results suggest that androgens and not sex chromosome genes play a predominant role in the masculinization of these behavioral and cognitive domains. To confirm this hypothesis, we compared brain activation during the performance of a 3D mental rotation task (MRT) in women with CAIS to control men and women (van Hemmen et al. 2016). We were able to recruit a total of 21 CAIS women, who were all gonadectomized and taking HRT (estrogens or combined estrogens/progestins) at the time of our study, and 30 control women and 30 control men. Groups were matched for age and educational level. The diagnosis of CAIS was based on both clinical characteristics and mutation analysis of the androgen receptor gene using genomic DNA. All participants received clear instructions for the MRT at the day of testing and performed a practice trial before the actual MRI session was started. The stimuli used were colored 3D objects (Shephard and Metzler 1971) with varying degrees of rotation ranging from 45° to 315°. The stimuli were presented in an alternating block design with five rotation or control trials in each block. For the control trails, the participants just had to answer the question whether an arrow was pointing to the left or to the right. Response latency (reaction time) and accuracy were recorded. Significant differences were observed with control men responding faster than women with CAIS, but there were no group differences in accuracy scores. At the neural level, an overall similar activation pattern during mental rotation after subtraction of activation during the control condition was found: all groups showed bilateral activations in the parietal lobe, predominantly in inferior and superior regions, extending into the occipital lobe. Significant activations were also observed in frontal areas, to a large extent in the precentral and superior and middle frontal gyrus. The overall pattern observed is consistent with results from a meta-analysis on neuroimaging studies during mental rotation (Zachs 2008). Between group analyses using region of interest (ROIs) revealed sex differences with control men showing significantly more activation than control women in the left inferior parietal lobe and a trend for the right inferior lobe as well. Women with CAIS resembled control women in neural activation patterns and thus differed significantly from control men. We found no significant correlations between circulating hormone levels (testosterone, estradiol) and brain activation patterns. These results thus suggest that reported sex differences in brain functioning while performing a spatial ability task is not directly driven by genetic sex but might be attributable to gonadal hormone exposure, most likely androgens. The absence of male-typical results in CAIS further supports the notion that in humans, androgens, and not estrogens, are the masculinizing hormones. However, we cannot distinguish between organizational and activational effects of these androgens as the insensitivity to androgens is already present prenatally and remains continuous throughout life. Furthermore, women with CAIS have supposedly higher serum estrogen levels than men due to aromatization of testosterone when

the gonads are still in situ (Hughes and Deeb 2006) and due to estrogen replacement therapy after gonadectomy. Thus the female-typical pattern in neural activation observed in CAIS can also be attributed to estradiol, which would be in line with studies that have proposed a role for estrogens in mental rotation-related neural activation and performance (e.g., Maki et al. 2002; Schöning et al. 2007), although it should be noted that others have not found these effects (e.g., Peters et al. 1995; Halari et al. 2005).

Finally, it cannot be ruled out that socialization has had an effect on brain activity as well. Exposure to typically masculine toys and activities is thought to have enhancing effects on performance on spatial tasks (e.g., Connor and Serbin 1977). Since recalled childhood toy and activity preferences were sex typical in our groups, i.e., there was a greater preference of masculine toys and activities in control men, and for feminine toys and activities in control women and women with CAIS. Gender stereotypes about male superiority on spatial tasks have also been proposed to affect the behavioral sex difference observed in the MRT (Hausmann et al. 2009). However, to minimize potential gender stereotype effects on the sex differences in neural activation, the participants were not informed about the sex difference in MRT performance before participation.

1.7.2 Brain Structure

Numerous neuroimaging studies have focused on macro- and mesoanatomical sex differences, such as in overall or regional gray (GM) and white matter (WM) volumes derived from structural MRI scans (for meta-analysis, see Ruigrok et al. 2014). Overall, it has been shown that men have larger bilateral GM volumes in limbic regions, including the amygdala, hippocampus, parahippocampal and cingulate gyrus, the temporal pole, precuneus, putamen, and cerebellum, whereas women have larger GM volumes in the bilateral thalamus and precuneus, right planum temporale/parietal operculum, insula, Heschl's and anterior cingulate gyrus, parts of the frontal cortex, and left parahippocampal gyrus and lateral occipital cortex. These sex differences are however not related to sex differences in total brain size (the male brain is on average 11% larger than the female brain). Evidence of early sex hormone effects on regional GM volumes seems to be rather inconsistent. In one study of boys (Lombardo et al. 2012), testosterone levels in amniotic fluid were associated with GM volume at age 8–11 in some, but not all brain regions showing sex differences. By contrast, no masculinizing effects of fetal testosterone on brain structure have been observed in girls diagnosed with CAH, although a decrease in amygdala volume was observed in both boys and girls with CAH which is probably caused by their glucocorticoid deficiency (Merke et al. 2003). Some effects of circulating gonadal hormones on adult GM volumes have been reported (e.g., Lessov-Schlaggar et al. 2005; Witte et al. 2010; Lentini et al. 2012), but results varied among studies probably due to methodological differences.

In a recent study (Savic et al. 2017), cortical thickness and subcortical GM volumes were compared between a group of 16 women diagnosed with CAIS and

control groups of men and women ($n = 32$/sex). It was found that both women with CAIS and control women displayed thicker parietal and occipital cortices and a thinner left temporal cortex than control men. Interestingly, women with CAIS also displayed a "male" pattern, i.e., a significantly thinner cortex in the precentral gyrus and to some extent, in the postcentral gyrus compared to female control women, and thus similar to control men. Furthermore, caudate volumes were significantly smaller, i.e., "male-like," in women with CAIS compared to control women, but hippocampus volumes were female-like and thus significantly larger than in control men. Thus women with CAIS showed a mixed male and female pattern suggesting direct effects of sex chromosome genes in addition to sex hormone effects.

The MRI technique of diffusion tensor imaging (DTI), which measures the diffusion of water molecules, was recently developed to study sex differences in white matter microstructure. The most used quantitative measure that can be derived from DTI is fractional anisotropy (FA) (Pierpaoli and Basser 1996). The FA value provides information about the degree of diffusion anisotropy. A low FA value reflects isotropic diffusion, i.e., equal diffusion in all directions, as, for instance, in cerebrospinal fluid. A high degree of anisotropy is found in WM fiber bundles, in which water diffusion is restricted in the direction perpendicular to the axon. The majority of DTI studies have found higher FA values in major WM regions in men compared to women (e.g., Chou et al. 2011; Inano et al. 2011; Schoonheim et al. 2014; Takao et al. 2014). Lower FA values in WM tracts have been observed in women with Turner syndrome (Holzapfel et al. 2006) and men with Klinefelter syndrome (DeLisi et al. 2005), but these two DSDs are characterized by sex chromosome aneuploidies and subsequent sex hormone deficiencies, which makes it impossible to determine the relative contribution of sex chromosomes versus sex hormones in the sexual differentiation of WM characteristics. Therefore, to address this particular question, we acquired DTI scans of women with CAIS and compared them with groups of control men and women (van Hemmen et al. 2017b). The final sample consisted of 20 women with CAIS, 30 control men, and 30 control women.

Using analyses based on tract-based spatial statistics, widespread sex differences were observed in FA with control men showing higher FA values than control women in a single cluster covering a large part of the skeleton, including major WM tracts; subcortical regions, such as the bilateral thalamus and basal ganglia; and the brain stem. By contrast, control women did not show any regions with higher FA values than control men. Similar differences were found between control men and women with CAIS with control men having higher FA values in a large part of the skeleton, whereas the reverse contrast did not reveal any significant differences. Furthermore, no differences in FA values were observed between control women and women with CAIS. These findings thus suggest a more important role for sex hormones, most likely masculinizing androgen and/or feminizing estrogen effects, than for genetic effects related to sex chromosome genes in the sexual differentiation of WM microstructure.

A limitation of these studies (Hamann et al. 2014; van Hemmen et al. 2016, 2017b; Savic et al. 2017) conducted in women with CAIS that no inferences can

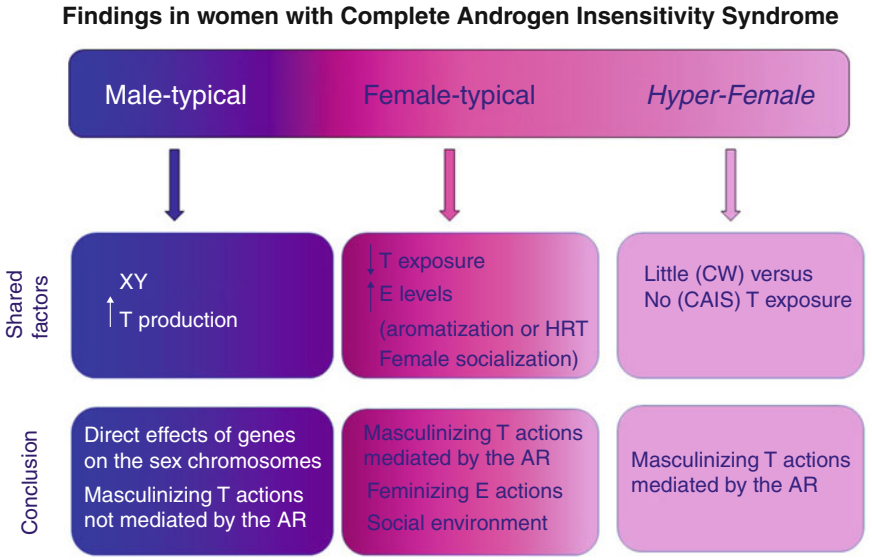

Fig. 1 Implications of male- and female-typical findings in women with CAIS. The upper boxes summarize the factors that women with CAIS share with the group of reference. In the lower boxes, the conclusions that can be drawn on these shared factors. *T* testosterone, *AR* androgen receptor, *E* estrogen, *HRT* hormone replacement therapy, *CW* control women, *CAIS* women with complete androgen insensitivity syndrome

be made about the exact timing of the proposed sex hormone effects. Since women with CAIS are insensitive to androgens throughout life, any finding obtained in adulthood can reflect organizational and/or activational sex hormone effects during the perinatal phase, adolescence, and/or adulthood. Longitudinal studies in women with CAIS, starting before adolescence, could provide important information regarding the timing of sex hormone effects on the sexual differentiation of the human brain.

To summarize, several sexually differentiated aspects of brain structure and function are female-typical in women with CAIS, although there is also evidence for male-typical neural characteristics. Female-typical findings in women with CAIS with respect to brain function were observed in the left inferior parietal lobe while performing a mental rotation task (van Hemmen et al. 2016) and in the amygdala when visualizing sexual images (Hamann et al. 2014). Regarding brain structure, a female-typical pattern was observed in regional GM volume of the hippocampus, as well as the parietal and occipital cortices (Savic et al. 2017), and in WM microstructure throughout extensive WM regions. By contrast, a male-typical caudate nucleus volume and pre- and postcentral gyrus cortex were observed in women with CAIS (Savic et al. 2017). These neuroimaging findings can be explained by several mechanisms (summarized in Fig. 1). Overall, neural development in the female direction in women with CAIS can be explained in three ways: (1) absence of masculinizing androgen effects following activation

of the AR, (2) by feminizing estrogen effects derived from aromatization of androgens, and (3) by female-typical socialization. By contrast, male-typical neural and behavioral characteristics might be explained by (1) sex chromosome effects and (2) masculinizing androgen effects not mediated by the AR.

1.7.3 Masculinizing Androgen Effects Mediated by the AR

Nonfunctional ARs, caused by genetic mutations in the AR gene, result in a lack of effective androgen exposure. Even though the production of testosterone in women with CAIS is within or above the male range when their gonads are still in situ (Melo et al. 2003; Doehnert et al. 2015), these androgens have no direct effect on target tissues, because they cannot activate the AR. Consequently, if a sexually differentiated aspect of brain structure or function is female-typical in women with CAIS, this might reflect an important role for masculinizing androgen effects through AR activation in the sex-typical development of these structures and functions. These proposed androgen effects could be organizational, activational, or both. In addition, the role of the AR in the masculinization of the adolescent brain has been studied by looking specifically at a functional polymorphism of the AR gene: a low number of CAG repeats has been associated with stronger androgen signaling and vice versa (Hsiao et al. 1999; Irvine et al. 2000). It was found to modulate relative GM and WM volumes (Paus et al. 2010), cortical thickness development (Raznahan et al. 2010), and WM growth (Perrin et al. 2008). It should be noted that long CAG repeats of the AR have also been associated with male-to-female transsexuality of which it has been hypothesized to reflect reduced androgen action during development (Hare et al. 2009).

Finally, since control women produce low amounts of androgens and have a functional AR, the level of androgen exposure differs between control women and women with CAIS. Therefore, in theory, the absence of any masculinizing androgen effects through the AR might result in "ultra-feminine" characteristics in women with CAIS (Fig. 1).

1.7.4 Masculinizing Androgen Effects Not Mediated by the AR

In general, it is difficult to determine whether potential androgen effects are the result of direct AR activation or indirect activation of the ER by androgen-derived estrogens upon aromatization, as is the predominant prenatal masculinizing pathway in rodent species (Bakker et al. 2006). It is, however, assumed that in humans masculinizing androgen effects are mediated by the AR and not the ER based on male-typical psychosexual development in men with aromatase deficiency or estrogen insensitivity due to a mutation in the estradiol receptor (Baum 2006), as well as on studies conducted in nonhuman primates (Wallen 2005). Previously reported predominant female-typical psychosexual characteristics in women with CAIS (Masica et al. 1971; Wisniewski et al. 2000; Hines et al. 2003; but see

T'Sjoen et al. 2011; Brunner et al. 2016) also argue against a critical role for estrogens in brain masculinization in humans. Interestingly, it should be noted that there is some evidence for an activational role for estradiol on the brain in adult men. Indeed, a large clinical study (Finkelstein et al. 2013) showed that treatment with an aromatase inhibitor led to a significant decline in sexual desire in adult men.

1.7.5 Feminizing Estrogen Effects

Women with CAIS are thought to have higher estrogen levels than men because androgens produced by the gonads can be aromatized to estrogens and following gonadectomy, women with CAIS generally take estrogens from puberty onwards to induce puberty (in case of prepubertal gonadectomy) and to maintain their health. Therefore, female-typical neural characteristics might reflect feminizing effects of estrogens.

Recent studies in mice have shown that female-typical neural and behavioral characteristics develop under the influence of estradiol during a specific prepubertal period (Brock et al. 2010, 2011). These results thus challenged the classical view of a default organization of the female brain. To date, in humans, there is only evidence for a role of testosterone (and not estradiol) in the development of the human brain as mentioned above (e.g., Baum 2006). However, there is some indirect evidence that estradiol might play a role in the development of the female brain. Several studies (Downey et al. 1989; Rolstad et al. 2007; Shaeffer et al. 2008) have shown that TS women reported that aspects of heterosexual function (e.g., ever engaging in genital petting or sexual intercourse, ever having had a boyfriend) were significantly lower compared to control women. Furthermore, Ross et al. (1998) reported some beneficial effects of treatment with lose doses of estrogen at prepubertal ages on cognitive function. Twenty-four TS girls exhibited a significant improvement in their motor function and nonverbal processing speed after estradiol treatment, when compared to their TS peers who received a placebo treatment. Clearly more research is needed to determine whether estradiol feminizes the brain in humans.

1.7.6 Socialization Effects

A major challenge when studying potential biological factors underlying the sexual differentiation of the human brain and behavior is that from the moment someone is born, his/her social environment is gender-biased. Since women with CAIS are raised as girls, they share a female-typical socialization with control women. Thus, female-typical findings in women with CAIS might reflect effects from the environment related to the gender of rearing. It has been thought that experience can alter the brain throughout life (e.g., Maguire et al. 2006), and gender-typical experiences might have an effect on sex differences found in brain structure and function. For example, exposure to male-typical toys and activities, such as playing action video games, might

result in better performance on spatial tasks (Connor and Serbin 1977; Feng et al. 2007). However, there is also a very strong suggestion of early androgen effects in toy preferences: studies in girls with CAH have shown that they were more interested in playing with male-typical instead of female-typical toys (Hines et al. 2016).

1.7.7 Sex Chromosome Effects

The presence of some male-typical neural characteristics in women with CAIS might be explained by the fact that they have a Y chromosome. Although hormone-independent effects of genes located on the sex chromosomes have long been overlooked as being relevant in brain sexual differentiation, recent animal studies have provided evidence for a role of sex chromosome genes in addition to sex hormone effects (e.g., Arnold and Chen 2009). Sexual differentiation as result of sex chromosome effects might reflect (1) direct effects of genes on the Y chromosome or (2) effects related to having one versus two X chromosomes. Studies in rodents have shown neural sex differences related to differences in *Sry* expression in the brain (Dewing et al. 2006). In individuals with two X chromosomes, one of the two X chromosomes is silenced to prevent higher expression of X-linked genes in XX versus XY cells (Chang et al. 2006). This silencing is referred to as X-inactivation and serves as a mechanism to reduce sex differences. However, the gene responsible for X-inactivation, *Xist*, is only expressed in XX cells and has now been suggested as a potential sex-differentiating gene (Arnold 2017). Approximately 10–15% of the X-linked genes escape X-inactivation (Carrel et al. 1999; Carrel and Willard 2005), of which some are located on the pseudoautosomal region (PAR), i.e., have Y-linked homologues, while others are outside the PAR (Disteche 2012). Higher expression of X-linked escapee genes located outside the PAR in XX versus XY cells may result in neural or behavioral sex differences. Furthermore, maternal versus paternal imprinting of the X chromosome might also influence sexual differentiation, as it might cause differences in expression of X-linked genes between men and women (Babak et al. 2015).

These proposed mechanisms of direct sex chromosome gene effects have not been adequately studied in humans yet, and even though animal studies have already provided some valuable information, many questions remain. A recent study using the FCG mouse model has shown that brain structure was related to sex hormone actions in 16, and sex hormone-independent effects in 11 brain regions (Corre et al. 2016) suggesting that the contribution of sex chromosome genes might still be underestimated (Arnold 2017). Human studies on cognitive abilities and brain structure and function in SCAs such as TS and KS have revealed a potential contribution of sex chromosome complement on, for example, verbal and spatial abilities and GM volume (reviewed in Printzlau et al. 2017), but these results remain difficult to interpret as they might also reflect sex hormone effects, since sex hormone levels are also affected in most SCAs.

2 Concluding Remarks

Sex differences exist in many aspects of human behavior, cognition, and brain structure and function. It is of great importance to identify these factors causing sex differences, not only to increase our understanding of the development of the healthy brain but also to provide valuable information on the ontogeny of several neuropsychiatric disorders with an important sex difference in their prevalence. Neuroimaging studies in women with CAIS suggest that sex differences in the human brain results from a combination of sex hormone-, sex chromosome-, and socialization-related effects and that the relative contribution of each factor might vary throughout the brain. Nevertheless, androgens acting through the AR seem to play a major role in inducing male-typical neural and psychosexual characteristics in humans. By contrast, whether female-typical neural and psychosexual characteristics develop under the influence of estrogens remains to be elucidated.

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