Origins of gender differences in the human brain

van Hemmen, J.

2017

document version
Publisher's PDF, also known as Version of record

Chapter 8

General discussion

In this thesis, studies in women with CAIS are described that had as their the objective to a) investigate the factors involved in the sexual differentiation of human brain structure and function, and b) study the link between proposed retrospective markers of early androgen levels and effective androgen exposure. In this chapter, the main findings of these studies are discussed, limitations are addressed, and directions for future research are proposed.

8.1 BRAIN STRUCTURE AND FUNCTION IN WOMEN WITH CAIS

We have shown that several sexually differentiated aspects of brain structure and function are female-typical in women with CAIS, although there are also indications that some of these female-typical findings are part of larger, spatially distributed patterns of neural characteristics that, overall, are neither male- nor female-typical. Female-typical findings in women with CAIS with respect to brain function were observed in the left inferior parietal lobe during mental rotation performance (Chapter 2), and with respect to brain structure, in regional GM volume of an area comprising the right pre- and postcentral gyrus (Chapter 4), and in WM microstructure throughout extensive WM regions, with the exception of AD-related WM characteristics (Chapter 3). Whereas these findings are based on mass-univariate analyses of differences between groups, a multivariate method was also employed for the analysis of neuroimaging data. Findings from whole-brain MPR analyses showed that the overall, spatially distributed patterns of WM microstructure and GM volume in women with CAIS were not similar to the patterns observed in female or male controls (Chapter 4 and 5).

With respect to the findings in regional GM volume and WM microstructure, it is important to focus on the differences between a mass-univariate versus an MPR analysis approach in more detail to understand why predominantly female-typical neural characteristics were found using mass-univariate analyses, whereas MPR analyses found patterns that were neither male- nor female-typical. When analyzing between-group differences using a mass-univariate approach, differences are assessed at the voxel level. This introduces a significant bias towards the detection of differences that are spatially localized (Davatzikos 2004). In contrast, classification algorithms generated with MPR methods rely on distributed instead of focal information, using many voxels simultaneously to search for regularities in the data associated with the variable of interest, such as gender. Consequently, this multivariate approach can detect subtle, spatially distributed patterns in the data (Schrouff et al. 2013). The findings from these different approaches therefore do not contradict, but rather complement each other, and might indicate that localized female-typical neural characteristics in women with CAIS are part of spatially distributed patterns of these characteristics that, overall, are not predominantly similar to those of control men or women.

8.2 IMPLICATIONS OF NEUROIMAGING FINDINGS IN WOMEN WITH CAIS

Our neuroimaging findings in adult women with CAIS can be explained by several mechanisms. These mechanisms provide information about specific factors that might be involved in the sexual differentiation of the brain in typical development (summarized in Fig. 1).

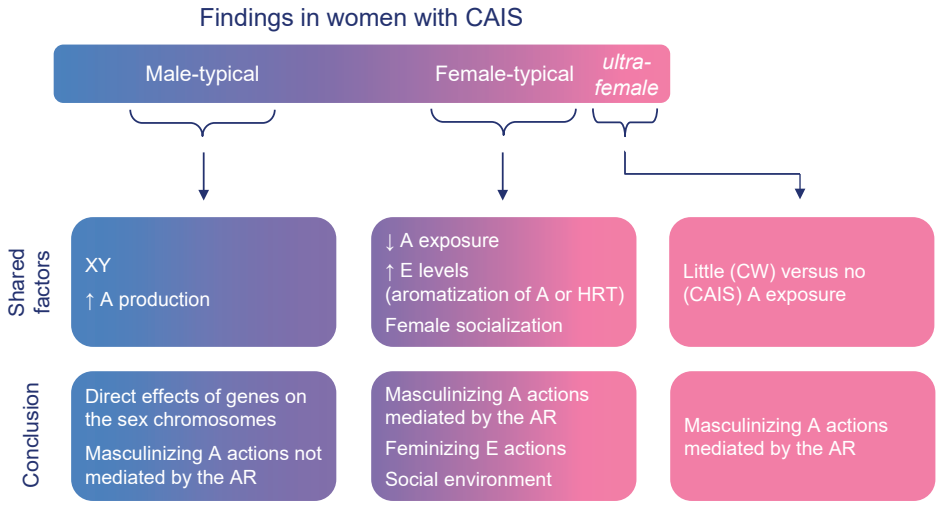

Figure 1 Implications of female- and male-typical findings in women with CAIS. The upper boxes summarize the factors that women with CAIS share with the group of reference. In the lower boxes, conclusions that can be drawn based on these shared factors are summarized. A = androgen, AR = androgen receptor, E = estrogen, HRT = hormone replacement therapy, CW = control women, CAIS = women with CAIS.

8.2.1 Female-typical neural characteristics in women with CAIS

Findings of female-typical spatial ability-related brain function (Chapter 2), regional GM volume (Chapter 4), and WM microstructure (Chapter 3) in women with CAIS described in this thesis, are complemented by a study demonstrating female-typical amygdala activation in response to viewing sexual images (Hamann et al. 2014). In general, neural development in the female direction in women with CAIS can be explained in three ways; by the absence of masculinizing androgen effects following activation of the AR, by feminizing estrogen effects, and by female-typical socialization.

8.2.1.1 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 *in situ* (Melo et al. 2003; Doehnert et al. 2015), these androgens have no direct influence on the target tissue, because they

cannot activate the AR. Consequently, if a sexually differentiated aspect of brain structure or function is feminine in women with CAIS, this might reflect an important role for masculinizing androgen effects by AR activation in the gender-typical development of these structures and functions. These proposed androgen effects may be organizational, activational, or both. Since control women produce low amounts of androgens and have functional ARs, there is a difference in androgen exposure between control women and women with CAIS. Therefore, in theory, the absence of masculinizing androgen effects through the AR might result in "ultra-feminine" characteristics in women with CAIS (see Fig. 1). These effects were not observed in the studies described in this thesis, although the methods we have used might not have had sufficient sensitivity to detect a difference between control women and women with CAIS reflecting differences in androgen exposure.

A majority of research on sexual differentiation of the brain and behavior in humans has focused on the role of androgens, with many studies concentrating on early organizational effects. For example, findings from studies in CAH, opposite-sex twins, and fetal testosterone levels measured in amniotic fluid have suggested that spatial abilities are influenced by early androgens (reviewed in Berenbaum and Beltz 2016). Although results from neuroimaging studies are less convincing, there are some findings supportive of androgen effects. For example, a study in boys with familial male precocious puberty (FMPP), characterized by early childhood androgen excess, has indicated a role for early androgens in GM development (Mueller et al. 2011), and fetal testosterone levels have been related to GM volume in certain sexually differentiated brain regions in boys (8-11 years; (Lombardo et al. 2012). Studies of GM and WM in CAH have, however, demonstrated similar differences between both men and women with CAH and controls, suggesting that these differences are likely to reflect other disease-related factors, such as prenatal glucocorticoid deficiency, instead of, or in addition to, early androgen excess (Merke et al. 2003; Speiser 2013). During puberty, increasing testosterone levels in boys have been linked to increases in WM volume (reviewed in Peper et al. 2011). Regarding activational androgen effects in adulthood, spatial abilities are thought to improve with increased testosterone levels in women and decreased testosterone levels in men (e.g., Aleman et al. 2004; Berenbaum et al. 2012). In addition, both positive and negative associations between brain structure and function, including MRT-related brain function, and circulating testosterone levels in adulthood have been found (e.g., Schöning et al. 2007; Mendrek et al. 2011; Heany et al. 2015). Some of these associations were located in sexually differentiated brain regions, although it is important to note that inconsistencies in findings exist, and not all studies have reported these associations.

In general, these studies do not allow to specify if potential androgen effects are the result of direct AR activation, or indirect ER activation by androgen-derived estrogens, as is the common prenatal masculinizing pathway in rodents. It is, however, assumed that in humans, masculinizing androgen effects are mediated by the AR based on male-typical psychosexual development in men with aromatase deficiency or estrogen insensitivity (Baum 2006) and

studies in non-human 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 of estrogenic masculinization in humans. In addition to these findings, the role of the AR in mediating adolescent androgen effects on human brain structure has been studied by the investigation of a functional polymorphism of the AR gene; a low number of CAG repeats is associated with stronger androgen signaling and vice versa (Hsiao et al. 1999; Irvine et al. 2000). Results have indicated AR-mediated effects of testosterone on relative GM and WM volumes (Paus et al. 2010), cortical thickness development (Raznahan et al. 2010), and WM growth (Perrin et al. 2008) during adolescence.

8.2.1.2 Feminizing estrogen effects

Women with CAIS are thought to have higher estrogen levels than men, because a) androgens produced by the gonads can be aromatized into estrogens (but see Doehnert et al. 2015), and b) after gonadectomy, women with CAIS generally use estrogen hormone replacement therapy (HRT) from puberty onwards to induce puberty (in case of prepubertal gonadectomy) and to maintain their health. Therefore, sexually differentiated neural characteristics in which women with CAIS resemble control women may reflect feminizing estrogen effects. Serum estradiol levels measured at the day of testing in our sample of women with CAIS were indeed higher than in control men and did not differ from control women (note that hormone levels were not obtained in the USA sample described in Chapter 4).

Associations between adult estrogen levels and MRT-related neural activation (Schöning et al. 2007), and between adult and adolescent estrogen levels and brain structure, including measures of GM and WM, have been described (e.g., reviewed in Peper et al. 2009; Catenaccio et al. 2016). Some of these findings are specifically related to areas of the brain that are sexually differentiated. Although it is difficult to summarize findings from these studies due to methodological differences, results seem to indicate that estrogens have the ability to exert effects on the brain during puberty and adulthood. These are likely transient, activational effects, but in puberty they might also have a role in brain organization (Sisk and Zehr 2005; Schulz et al. 2009). Whether there is an active, feminizing role for estrogens during early brain development in humans, as has been suggested in mice (Brock et al. 2011), is currently unknown.

8.2.1.3 Socialization effects

One of the challenges in understanding the biological factors underlying human sexual differentiation is the fact that, from the moment of birth, a person's social environment is gender-biased. Since women with CAIS are raised as girls, they share female-typical socialization with control women. Thus, female-typical findings in women with CAIS might reflect environmental influences related to gender-of-rearing.

In general, experience can alter the brain throughout life (e.g., Maguire et al. 2006), and gender-typical experiences might have an influence on the gender differences found in brain structure and function. For instance, exposure to masculine toys and activities, such as playing action video games, might result in better performance on spatial tasks (Connor and Serbin 1977; Baenninger and Newcombe 1989; Feng et al. 2007). Although gender-typed toy and activity preferences are generally thought to result from both early androgen (Hines 2011) and socialization effects, a recent study has indicated that these effects might not be independent (Hines et al. 2016). In this study, girls with CAH showed reduced self-socialization of gendered behavior, i.e., were less susceptible to information about the appropriateness of toys and objects for girls, suggesting a role for early androgens in later self-socialization. Therefore, socialization effects on brain sexual differentiation might be related to early androgen effects.

8.2.2 Non-female-typical neural characteristics in women with CAIS

Whole-brain spatially distributed patterns of GM volume and WM microstructure did not appear female-typical in women with CAIS (Chapter 4 and 5). These neuroimaging studies in women with CAIS suggest a deviation from overall neural development in the female direction. Similarly, a behavioral study on spatial learning and memory found no significant differences between women with CAIS and control men and women, while effect sizes were suggestive of a more female-like performance on some, but not all variables assessed (Mueller et al. 2016). Even though these non-female-typical findings were not male-typical either, it is important to explore the mechanisms that could explain male-typical findings in women with CAIS for a correct interpretation of these outcomes. Only a combination of mechanisms related to male-typical findings and previously described factors related to female neural development in women with CAIS might explain neural characteristics that are neither male- nor female-typical.

8.2.2.1 Sex chromosome gene effects

Having an XY chromosome pair is an important factor that women with CAIS have in common with control men. Although hormone-independent effects of genes located on the sex chromosomes have long been overlooked as a factor of relevance 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; Cox et al. 2014; Corre et al. 2016). Sexual differentiation as a result of sex chromosome effects might reflect a) direct effects of genes on the Y chromosome, or b) effects related to having one versus two X chromosomes. For example, studies in rodents have demonstrated neural sex differences related to differences in Sry expression in the brain (Dewing et al. 2006). In XX chromosome pairs, 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 gender 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 located outside the PAR region (Disteche 2012). Higher expression of X-linked escapee genes located outside the PAR in XX versus XY cells may result in brain or behavioral gender differences. 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, although animal studies have already provided valuable information, many questions remain. Based on a recent study using the FCG model in mice, demonstrating that brain structure was related to sex hormone actions in 16, and sex hormone-independent sex chromosome effects in 11 brain regions (Corre et al. 2016), it has been suggested that the contribution of the sex chromosomes might still be underestimated as a result of the large amount of studies on sex hormone effects (Arnold 2017). Human studies on cognitive abilities and brain structure and function in SCAs such as TS and KS, have revealed a potential impact of factors related to sex chromosome complement on, for example, verbal and spatial abilities and GM volume (e.g., reviewed in Printzlau et al. 2017). However, these results are difficult to interpret, as they might also reflect sex hormone effects, because sex hormone levels are affected in most SCAs.

8.2.2.1 Masculinizing androgen effects not mediated by the AR

The production of high levels of androgens prior to gonadectomy presents a second factor that women with CAIS have in common with control men. Although these androgens cannot activate the AR in women with CAIS, they can have indirect effects that are not mediated by the AR. In rodents, the main route for early androgen effects on male-typical brain organization is through aromatization into estrogens and subsequent activation of the ER (reviewed in Baum 1979). As explained in section 8.2.1.1, there are currently no indications for masculinizing androgen effects through the ER in humans, although direct evidence against it does not exist either. Therefore, if masculinizing actions mediated by the ER do play a role in humans, these effects would result in neural masculinization in women with CAIS.

8.2.3 Conclusions on neuroimaging findings in women with CAIS

Our female-typical findings in women with CAIS with respect to localized brain activation related to spatial cognition (Chapter 2) and brain structure, namely regional GM volume (Chapter 4) and WM microstructure (Chapter 3), indicate that gender differences in these

neural characteristics reflect sex hormone effects and/or female-typical socialization. More specifically, these sex hormone effects might result from masculinizing androgen actions mediated by the AR and/or feminizing estrogen actions. Based on current findings, it is unclear when in life these hormone effects occur and whether their effects are organizational, activational, or both. Our findings also argue against a prominent role of sex chromosome genes and masculinizing androgen effects through the ER in sexual differentiation of these neural characteristics in reported brain regions.

The results from MPR analyses, showing that women with CAIS were not more likely to be classified as control women than as control men based on spatially distributed whole-brain patterns of GM volume and WM microstructure (Chapter 4 and 5), may suggest that these sex hormone and/or socialization effects do not dominate sexual differentiation throughout the entire brain. Instead, in sexually differentiated brain regions, or patterns of brain structure that are not female-typical in women with CAIS (at least with respect to GM volume and WM microstructure), other factors might also play a role in brain sexual differentiation; these may be direct effects of genes on the sex chromosomes and/or masculinizing androgen actions not mediated by the AR. The relative influence of these factors throughout the brain cannot be inferred from the current findings. However, based on the absence of male-typical localized findings in women with CAIS in the studies performed to date, it can be speculated that there are no specific brain regions in which direct sex chromosome effects, or masculinizing androgen effects not mediated by the AR, have a pivotal influence on brain structure and function. Instead, it is more likely that multiple factors interact, or have additive effects throughout sexually differentiated brain regions that are not female-typical in women with CAIS. However, we cannot rule out the possibility that future studies on other sexually differentiated aspects of the brain, or in larger samples of women with CAIS, will find neural characteristics more similar to control men, since animal studies have shown brain regions with prominent sex hormone-independent effects of sex chromosome genes on sexual differentiation (Corre et al. 2016).

Taken together, neuroimaging findings in women with CAIS suggest that brain sexual differentiation in humans is multifactorial and is likely to include a combination of sex hormone, sex chromosome, and socialization effects, with each one contributing in varying degrees throughout the brain. This conclusion is in line with the recently proposed general theory of sexual differentiation, which focuses on biological mechanisms, i.e. sex hormones and sex chromosomes, but also acknowledges a role for social environment and experience (Arnold 2017).

8.3 POTENTIAL RETROSPECTIVE MARKERS OF EARLY ANDROGEN LEVELS

The timing of the proposed sex hormone effects is unknown and presents one of the limitations of the neuroimaging studies in women with CAIS described in this thesis, since all studies were performed in adults (see section 8.4). Because retrospective markers of early androgen levels have the potential to provide additional information about the timing of effects in these studies, we assessed the validity of two proposed markers of early androgen levels, CEOAEs and 2D:4D ratios, by comparing these measures between women with CAIS and control men and women (Chapter 6). There were some indications for androgen effects on both measures; a tendency towards female-like right-ear CEOAE amplitudes and significant female-typical right-hand 2D:4D ratios in women with CAIS. However, the within-group variability in women with CAIS was not lower than in control groups, which would be expected if both measures would solely reflect effective androgen exposure. These findings can only be explained if other, non-androgen factors, also contribute to these measures obtained in adulthood. Therefore, using these measures as retrospective markers is not recommended, since they cannot be assumed to provide a reliable indication of early androgen levels.

The digit ratio results presented in this thesis are a replication of a previous study of digit ratios in women with CAIS (Berenbaum et al. 2009). The authors of this previous study have also interpreted their findings as evidence against the validity of 2D:4D ratios as a marker of early androgen levels. Nonetheless, studies have continued to use digit ratios for this purpose, while citing the study by Berenbaum et al. (2009) as affirmative. The usage of invalid markers of early androgen exposure might, unfortunately, result in spurious associations with the variables of interest.

The anogenital distance (AGD) might present a promising biomarker of early androgen levels. AGD is sexually differentiated at birth, and findings from both experimental animal studies and observational human studies are supportive of a link between AGD at birth and prenatal androgen exposure (reviewed in Thankamony et al. 2016), while the postnatal androgen surge in boys might also influence AGD development during the first months of life (Thankamony et al. 2009; Mitchell et al. 2015). In order to assess the validity of this measure as a marker of early androgen levels, future studies in humans are warranted to establish a direct link between AGD at birth and prenatal androgen exposure, and also to investigate to what extent AGD is affected by postnatal hormones, and how stable this measure is throughout life.

8.4 LIMITATIONS AND DIRECTIONS FOR FUTURE RESEARCH

The results from this thesis are of great importance in increasing our understanding of the mechanisms underlying brain sexual differentiation in humans, but should be interpreted in the light of several limitations and the need for further research.

Even though the study design used in this thesis provided novel insights into the factors involved in brain sexual differentiation, and might have identified effects that could not have been determined previously, there are some limitations regarding the specificity of the results. First, we were unable to differentiate between the potential mechanisms of action related to either male- or female-typical findings in women with CAIS (see Fig. 1). For example, the female-typical findings presented in this thesis are highly informative because they provide evidence against a direct prominent influence of genes on the sex chromosomes and masculinizing androgen actions that are not AR-mediated in sexual differentiation of these specific neural characteristics in humans. However, further specification of the mechanisms that might explain these findings, i.e., androgenic masculinization through AR activation, estrogenic feminization, and/or socialization, is not possible based on the study design used in this thesis. Therefore, whether these neural characteristics are primarily under the influence of one, or a combination of two or more of these factors, remains to be determined. According to previous research, there might be a role for all three mechanisms, but this conclusion cannot be drawn based on the results from our studies in adult women with CAIS. Regarding the findings in women with CAIS that were neither male- nor female-typical, conclusions were even less specific in terms of identifying the exact mechanisms of action. None of the potential gender-biasing factors, i.e., AR-mediated, or not AR-mediated androgenic masculinization, estrogenic feminization, sex chromosome gene effects, and/or socialization-related effects, could be ruled out in this situation. Related to this issue is the general challenge in human studies to separate biological from environmental influences, but, as discussed in section 8.2.1.3, these factors might not necessarily be independent of each other.

Second, the timing and permanence of the proposed sex hormone effects could not be determined using our study design. For example, major androgen effects may occur during the prenatal phase, early postnatal life, puberty, and adulthood. In addition, these effects can be permanent organizational, or transient activational. Since our subjects were all adults, and androgen insensitivity is present throughout life including the prenatal phase, we cannot draw any definite conclusions on when sexual differentiation of the brain occurs under the influence of androgens. This is also true for potential feminizing estrogen effects in women with CAIS, since they are thought to have higher estrogen levels than control men when their gonads are *in situ* and receive female levels of estrogens as hormone replacement after gonadectomy.

Future efforts should be aimed at overcoming these first two limitations in order to be able to further specify the mechanisms underlying sexual differentiation of the human brain. Several adaptations to the study design used in this thesis might enable this. First, when studying adult women with CAIS who have been gonadectomized, it would be interesting to test the effects of estrogen replacement by obtaining neuroimaging data both on and off HRT. This might provide a better understanding of potential feminizing activational effects of estrogens on the brain. One important aspect to consider is whether such a design would be ethically justified, as estrogen replacement in women with CAIS is necessary to maintain their health. Second, longitudinal studies in women with CAIS, starting before puberty, should be able to further identify the timing of purported hormone effects. Third, as discussed in section 8.3, identification of a valid retrospective biomarker of early androgen exposure would enable the assessment of early androgen effects. This would be of great value for the research field focusing on brain and behavioral sexual differentiation as a whole, and also specifically for studies in women with CAIS as it would enable inferences regarding the influence of effective androgen exposure during early development.

A third limitation of the studies in the present project is related to the functional significance of the observed neural gender differences. It is challenging to draw conclusions about the functional implications of these findings with respect to specific behavioral and cognitive gender differences. Although with task-fMRI the association between neural activation and task performance can be assessed, the interpretation of, for instance, differences in regional GM volume in terms of behavior is not straightforward. While it might be tempting to draw conclusions based on the assumption that a structural gender difference in a brain region known to be involved in a certain cognitive ability is also functionally related to the behavioral gender difference, empirical testing is required to establish this link (e.g., McCarthy 2016; Tunç et al. 2016). Another important aspect to consider is that not all gender differences in brain structure and function necessarily result in behavioral gender differences. Men and women may have evolved different compensational strategies to reach the same endpoint, such as a certain behavior, regardless of differences in gender-biasing mechanisms (De Vries 2004; Arnold 2014). One illustrative example is that, even though the brains of men and women differ in overall size, this does not result in a gender difference in intelligence. It has, therefore, been suggested that females use a compensational mechanism, i.e., use their smaller brains more effectively, to overcome this difference (Grabowska 2017). Neural activation related to mental rotation performance has also been suggested to reflect different strategies in men and women, even if groups were matched on task performance (Jordan et al. 2002).

Finally, a general methodological limitation is that interpretation of the factors involved in the sexual differentiation of the brain based on neuroimaging findings in women with CAIS is only possible if previously reported gender differences are replicated in the control groups. For example, in Chapter 5, not all neuroimaging modalities had sufficient predictive

value to discriminate control men from control women, therefore prohibiting interpretation of the data in women with CAIS. Increasing sample sizes might increase the power to detect gender differences. Related to this, increasing the sample of women with CAIS might increase the power to detect differences between women with CAIS and control groups. This would provide a further understanding and localization of brain structure and function under the influence of sex hormones, sex chromosome genes, and/or gender-specific socialization. Since CAIS is a rare syndrome, large international research consortia and collaborations are required in order to achieve this.

CONCLUSION

Studies in women with CAIS provide the unique opportunity to investigate the mechanisms underlying human sexual differentiation. This thesis, comprising several studies in women with CAIS, presents the first extensive investigation of the relative contribution of sex hormones and sex chromosome genes to the sexual differentiation of several aspects of human brain structure and function. Our neuroimaging findings suggest that gender differences in the human brain are the result of multiple factors, most likely a combination of sex hormone, sex chromosome, and socialization-related effects. In addition, it was found that the relative contribution of each factor might vary throughout the brain. To further investigate to what extent sex hormone exposure during the prenatal period contributes to brain sexual differentiation, valid retrospective markers of prenatal hormone levels are required. Our assessment of the 2D:4D ratio and CEOAEs in women with CAIS showed that these measures obtained in adulthood do not provide a sufficiently reliable indication of early androgen exposure to be used for this purpose, and future work is needed to validate other potential biomarkers.

REFERENCES

Aleman A, Bronk E, Kessels RPC, Koppeschaar HPF, van Honk J. 2004. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology. 29:612–617.

Arnold AP. 2014. Conceptual frameworks and mouse models for studying sex differences in physiology and disease: Why compensation changes the game. Exp Neurol. 259:2–9.

Arnold AP. 2017. A general theory of sexual differentiation. J Neurosci Res. 95:291–300.

Arnold AP, Chen X. 2009. What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? Front Neuroendocrinol. 30:1–9.

Babak T, DeVeale B, Tsang EK, Zhou Y, Li X, Smith KS, Kukurba KR, Zhang R, Li JB, van der Kooy D, Montgomery SB, Fraser HB. 2015. Genetic conflict reflected in tissue-specific maps of genomic imprinting in human and mouse. Nat Genet. 47:544–549.

Baenninger M, Newcombe N. 1989. The role of experience in spatial test performance: A meta-analysis. Sex Roles. 20:327–344.

Baum MJ. 1979. Differentiation of coital behavior in mammals: A comparative analysis. Neurosci Biobehav Rev. 3:265–284.

Baum MJ. 2006. Mammalian animal models of psychosexual differentiation: When is "translation" to the human situation possible? Horm Behav. 50:579–588.

Berenbaum SA, Beltz AM. 2016. How early hormones shape gender development. Curr Opin Behav Sci. 7:53–60.

Berenbaum SA, Bryk KK, Nowak N, Quigley CA, Moffat S. 2009. Fingers as a marker of prenatal androgen exposure. Endocrinology. 150:5119–5124.

Berenbaum SA, Bryk KLK, Beltz AM. 2012. Early androgen effects on spatial and mechanical abilities: evidence from congenital adrenal hyperplasia. Behav Neurosci. 126:86–96.

Brock O, Baum MJ, Bakker J. 2011. The development of female sexual behavior requires prepubertal estradiol. J Neurosci. 31:5574–5578.

Brunner F, Fliegner M, Krupp K, Rall K, Brucker S, Richter-Appelt H. 2016. Gender Role, Gender Identity and Sexual Orientation in CAIS ("XY-Women") Compared With Subfertile and Infertile 46,XX Women. J Sex Res. 53:109–124.

Carrel L, Cottle AA, Goglin KC, Willard HF. 1999. A first-generation X-inactivation profile of the human X chromosome. Proc Natl Acad Sci U S A. 96:14440–14444.

Carrel L, Willard HF. 2005. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature. 434:400–404.

Catenaccio E, Mu W, Lipton ML. 2016. Estrogen- and progesterone-mediated structural neuroplasticity in women: evidence from neuroimaging. Brain Struct Funct. 221:3845–3867.

Chang SC, Tucker T, Thorogood NP, Brown CJ. 2006. Mechanisms of X-chromosome inactivation. Front Biosci. 11:852–866.

Connor JM, Serbin LA. 1977. Behaviorally Based Masculine- and Feminine-Activity-Preference Scales for Preschoolers: Correlates with Other Classroom Behaviors and Cognitive Tests. Child Dev. 48:1411–1416.

Corre C, Friedel M, Vousden DA, Metcalf A, Spring S, Qiu LR, Lerch JP, Palmert MR. 2016. Separate effects of sex hormones and sex chromosomes on brain structure and function revealed by high-resolution magnetic resonance imaging and spatial navigation assessment of the Four Core Genotype mouse model. Brain Struct Funct. 221:997–1016.

Cox KH, Bonthuis PJ, Rissman EF. 2014. Mouse model systems to study sex chromosome genes and behavior: relevance to humans. Front Neuroendocrinol. 35:405–419.

Davatzikos C. 2004. Why voxel-based morphometric analysis should be used with great caution when characterizing group differences. Neuroimage.

De Vries GJ. 2004. Minireview: Sex Differences in Adult and Developing Brains: Compensation, Compensation, Compensation. Endocrinology. 145:1063–1068.

Dewing P, Chiang CWK, Sinchak K, Sim H, Fernagut P-O, Kelly S, Chesselet M-F, Micevych PE, Albrecht KH, Harley VR, Vilain E. 2006. Direct Regulation of Adult Brain Function by the Male-Specific Factor SRY. Curr Biol. 16:415–420.

Disteche CM. 2012. Dosage Compensation of the Sex Chromosomes. Annu Rev Genet. 46:537–560.

Doehnert U, Bertelloni S, Werner R, Dati E, Hiort O. 2015. Characteristic features of reproductive hormone profiles in late adolescent and adult females with complete androgen insensitivity syndrome. Sex Dev. 9:69–74.

Feng J, Spence I, Pratt J. 2007. Playing an Action Video Game Reduces Gender Differences in Spatial Cognition. Psychol Sci. 18:850–855.

Grabowska A. 2017. Sex on the brain: Are gender-dependent structural and functional differences associated with behavior? J Neurosci Res. 95:200–212.

Hamann S, Stevens J, Vick JH, Bryk K, Quigley CA, Berenbaum SA, Wallen K. 2014. Brain responses to sexual images in 46,XY women with complete androgen insensitivity syndrome are female-typical. Horm Behav. 66:724–730.

Heany SJ, van Honk J, Stein DJ, Brooks SJ. 2015. A quantitative and qualitative review of the effects of testosterone on the function and structure of the human social-emotional brain. Metab Brain Dis.

Hines M. 2011. Gender development and the human brain. Annu Rev Neurosci. 34:69–88.

Hines M, Ahmed SF, Hughes IA. 2003. Psychological outcomes and gender-related development in complete androgen insensitivity syndrome. Arch Sex Behav. 32:93–101.

Hines M, Pasterski V, Spencer D, Neufeld S, Patalay P, Hindmarsh PC, Hughes IA, Acerini CL. 2016. Prenatal androgen exposure alters girls' responses to information indicating gender-appropriate behaviour. Philos Trans R Soc B Biol Sci. 371:20150125.

Hsiao PW, Lin DL, Nakao R, Chang C. 1999. The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem. 274:20229–20234.

Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA. 2000. Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet. 9:267–274.

Jordan K, Wüstenberg T, Heinze HJ, Peters M, Jäncke L. 2002. Women and men exhibit different cortical activation patterns during mental rotation tasks. Neuropsychologia. 40:2397–2408.

Lombardo M V, Ashwin E, Auyeung B, Chakrabarti B, Taylor K, Hackett G, Bullmore ET, Baron-Cohen S. 2012. Fetal testosterone influences sexually dimorphic gray matter in the human brain. J Neurosci. 32:674–680.

Maguire EA, Woollett K, Spiers HJ. 2006. London taxi drivers and bus drivers: A structural MRI and neuropsychological analysis. Hippocampus. 16:1091–1101.

Masica DN, Money J, Ehrhardt AA. 1971. Fetal feminization and female gender identity in the testicular feminizing syndrome of androgen insensitivity. Arch Sex Behav. 1:131–142.

McCarthy MM. 2016. Multifaceted origins of sex differences in the brain. Philos Trans R Soc London B Biol Sci. 371:20150106.

Melo KFS, Mendonca BB, Billerbeck AEC, Costa EMF, Inácio M, Silva FAQ, Leal AMO, Latronico AC, Arnhold IJP. 2003. Clinical, hormonal, behavioral, and genetic characteristics of androgen insensitivity syndrome in a Brazilian cohort: five novel mutations in the androgen receptor gene. J Clin Endocrinol Metab. 88:3241–3250.

Mendrek A, Lakis N, Jiménez J. 2011. Associations of sex steroid hormones with cerebral activations during mental rotation in men and women with schizophrenia. Psychoneuroendocrinology. 36:1422–1426.

Merke DP, Fields JD, Keil MF, Vaituzis AC, Chrousos GP, Giedd JN. 2003. Children with classic congenital adrenal hyperplasia have decreased amygdala volume: potential prenatal and postnatal hormonal effects. J Clin Endocrinol Metab. 88:1760–1765.

Mitchell RT, Mungall W, McKinnell C, Sharpe RM, Cruickshanks L, Milne L, Smith LB. 2015. Anogenital Distance Plasticity in Adulthood: Implications for Its Use as a Biomarker of Fetal Androgen Action. Endocrinology. 156:24–31.

Mueller SC, Merke DP, Leschek EW, Fromm S, VanRyzin C, Ernst M. 2011. Increased medial temporal lobe and striatal grey-matter volume in a rare disorder of androgen excess: a voxel-based morphometry (VBM) study. Int J Neuropsychopharmacol. 14:445–457.

Mueller SC, Verwilst T, Van Branteghem A, T'Sjoen G, Cools M. 2016. The contribution of the androgen receptor (AR) in human spatial learning and memory: A study in women with complete androgen insensitivity syndrome (CAIS). Horm Behav. 78:121–126.

Paus T, Nawaz-Khan I, Leonard G, Perron M, Pike GB, Pitiot A, Richer L, Susman E, Veillette S, Pausova Z. 2010. Sexual dimorphism in the adolescent brain: Role of testosterone and androgen receptor in global and local volumes of grey and white matter. Horm Behav. 57:63–75.

Peper JS, Brouwer RM, Schnack HG, van Baal GC, van Leeuwen M, van den Berg SM, Delemarre-Van de Waal HA, Boomsma DI, Kahn RS, Hulshoff Pol HE. 2009. Sex steroids and brain structure in pubertal boys and girls. Psychoneuroendocrinology. 34:332–342.

Peper JS, Hulshoff Pol HE, Crone EA, van Honk J. 2011. Sex steroids and brain structure in pubertal boys and girls: a mini-review of neuroimaging studies. Neuroscience. 191:28–37.

Perrin JS, Hervé P-Y, Leonard G, Perron M, Pike GB, Pitiot A, Richer L, Veillette S, Pausova Z, Paus T. 2008. Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. J Neurosci. 28:9519–9524.

Printzlau F, Wolstencroft J, Skuse DH. 2017. Cognitive, behavioral, and neural consequences of sex chromosome aneuploidy. J Neurosci Res. 95:311–319.

Raznahan A, Lee Y, Stidd R, Long R, Greenstein D, Clasen L, Addington A, Gogtay N, Rapoport JL, Giedd JN. 2010. Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. Proc Natl Acad Sci. 107:16988–16993.

Schöning S, Engelien A, Kugel H, Schäfer S, Schiffbauer H, Zwitserlood P, Pletziger E, Beizai P, Kersting A, Ohrmann P, Greb RR, Lehmann W, Heindel W, Arolt V, Konrad C. 2007. Functional anatomy of visuo-spatial working memory during mental rotation is influenced by sex, menstrual cycle, and sex steroid hormones. Neuropsychologia. 45:3203–3214.

Schrouff J, Rosa MJ, Rondina JM, Marquand AF, Chu C, Ashburner J, Phillips C, Richiardi J, Mourão-Miranda J. 2013. PRoNTo: pattern recognition for neuroimaging toolbox. Neuroinformatics. 11:319–337.

Schulz KM, Molenda-Figueira H a, Sisk CL. 2009. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. Horm Behav. 55:597–604.

Sisk CL, Zehr JL. 2005. Pubertal hormones organize the adolescent brain and behavior. Front Neuroendocrinol. 26:163–174.

Speiser PW. 2013. Congenital Adrenal Hyperplasia: Neuroendocrine, Behavioral and Cognitive Implications. In: Pfaff D,, Christen Y, editors. Multiple origins of sex differences in brain, Neuroendocrine functions and their pathologies. Springer Berlin Heidelberg. p. 71–79.

T'Sjoen G, De Cuypere G, Monstrey S, Hoebeke P, Freedman FK, Appari M, Holterhus P-M, Van Borsel J, Cools M. 2011. Male Gender Identity in Complete Androgen Insensitivity Syndrome. Arch Sex Behav. 40:635–638.

Thankamony A, Ong K, Dunger D, Acerini C, Hughes I. 2009. Anogenital Distance from Birth to Two Years: a Population Study. Environ Health Perspect. 117:1786–1790.

Thankamony A, Pasterski V, Ong KK, Acerini CL, Hughes IA. 2016. Anogenital distance as a marker of androgen exposure in humans. Andrology. 4:616–625.

Tunç B, Solmaz B, Parker D, Satterthwaite TD, Elliott MA, Calkins ME, Ruparel K, Gur RE, Gur RC, Verma R. 2016. Establishing a link between sex-related differences in the structural connectome and behaviour. Philos Trans R Soc B Biol Sci. 371:20150111.

Wallen K. 2005. Hormonal influences on sexually differentiated behavior in nonhuman primates. Front Neuroendocrinol. 26:7–26.

Wisniewski AB, Migeon CJ, Meyer-Bahlburg HF, Gearhart JP, Berkovitz GD, Brown TR, Money J. 2000. Complete androgen insensitivity syndrome: long-term medical, surgical, and psychosexual outcome. J Clin Endocrinol Metab. 85:2664–2669.