INTRODUCTION

The transgender population referred for gender affirming therapy has been steeply increased in the last decades [1]. A large part of the transgender population desires hormone therapy. Sex steroid administration in transgender individuals induces physical characteristics of the experienced gender via the binding to sex steroid receptors. One of the organs that is highly sensitive for sex steroids is the brain [2,3]. Sex steroids probably have organizational effects as well as activational effects on the brain [4]. Organizational effects of sex steroids play a role in the development sex-typical brain characteristics. It is hypothesized that perturbations in the fetal sex steroid milieu can lead to permanent sex-atypical brain characteristics and to gender dysphoria [5]. Hormone therapy in transgender individuals possibly induces activational effects: reversible alterations in physiological and pathological brain processes. While the number of studies in the transgender population is growing, there is still little known about the transgender brain. In this thesis we had two aims. The first aim was finding support for the hypothesis that early sex steroid perturbations may have led to a sex-atypical brain organization in transgender individuals. Therefore, we examined the neurobiological characteristics of transgender individuals prior to hormone therapy with resting-state functional magnetic resonance imaging (part 1). The second aim was to get more insight into the (activational) effects of transgender hormone therapy on physiological and pathological brain processes by investigating pituitary functioning (part 2.1), benign brain tumor risk, and stroke risk in transgender individuals receiving exogenous sex steroids (part 2.2).

PART 1 – NEUROBIOLOGY OF GENDER DYSPHORIA

To date, many transgender individuals and their relatives, are still waiting for a neurobiological underpinning of gender dysphoria. While literature about this topic is scarce, several studies provide support for the hypothesis that transgender individuals have experienced a sex-atypical brain organization (see review of Smith et al. [6]). However, few
recent studies found new additional explanations regarding the neurobiology of gender dysphoria. These studies found brain features in transgender individuals that were different from cisgender men as well as cisgender women, and thus gender dysphoria-specific. They hypothesized that these gender dysphoria-specific brain features are associated with the subjective experiences of incongruence between the gender identity and the sex assigned at birth [7–10]. One method to examine the neurobiological aspects of gender dysphoria, is to visualize brain resting-state networks. In chapter 4 of this thesis we compared functional connectivity patterns within four well-known resting-state networks (default mode network, salience network, and left and right working memory network) between adult transwomen and transmen using gonadotropin-releasing hormone analogues but not exogenous sex steroids, and cisgender men and cisgender women. In only one of these networks (the right working memory network) we found a difference in functional connectivity among groups. Cisgender men showed significantly greater functional connectivity in the right caudate nucleus than cisgender women. No such sex difference in functional connectivity was found in the transgender groups. Four months of transgender hormone therapy did not affect these results. These data may suggest that cisgender men and cisgender women have experienced a dissimilar differentiation of the right working memory network, and that such differentiation is less pronounced in transgender individuals. However, although endogenous sex steroid concentrations showed no relation with resting-state functional connectivity in cisgender participants, we cannot exclude that our findings are provoked by the use of gonadotropin-releasing hormone analogues in the transgender participants.

In chapter 5 of this thesis we focused on transgender youth. We compared functional connectivity patterns among prepubertal transgirls, prepubertal transboys, prepubertal cisgender boys, and prepubertal cisgender girls, and among adolescent transgirls, adolescent transboys, adolescent cisgender boys, and adolescent cisgender girls. In contrast to the other
groups, the transgender adolescents used puberty suppressors. Based on more recent available literature we included some other resting-state networks in chapter 5 than in chapter 4: visual networks, sensorimotor networks, default mode network, and the salience network. We found that within one of the three visual networks (visual network-I), adolescent transgirls showed greater functional connectivity in the right cerebellum than all other adolescent groups (thus a gender dysphoria-specific pattern). A sex difference in functional connectivity in the cisgender adolescent group was observed in the right supplementary motor area within one of the two sensorimotor networks (sensorimotor network-II), and in the right posterior cingulate gyrus within the posterior part of the fragmented default mode network. Within these networks adolescent transgirls showed functional connectivity patterns similar to their experienced gender (thus sex-atypical). Adolescent transboys showed a functional connectivity pattern similar to their experienced gender (thus sex-atypical) within the sensorimotor network-II only. The prepubertal children did not show any group differences in functional connectivity. That prepubertal transgender children, in contrast to transgender adolescents, did not show a gender dysphoria-specific pattern within visual network-I may be explained by the higher rate of inhomogeneity of this group (in many prepubertal children, gender dysphoria dissolves during puberty [11]). That prepubertal cisgender children, in contrast to cisgender adolescents, did not show sex differences in any of the networks may be explained by their younger age or more immature brain as sex-dimorphic brain patterns may arise during or after puberty [12–14]. The sex difference in the default mode network that was detected in adolescents was not found in adults. However, it is difficult to compare these findings as the default network was fragmented in the adolescent participants but not in the adult participants [14].

In summary, we found evidence for a neurobiological basis of gender dysphoria. We found less sex-typical and sex-atypical brain features in transgender individuals which support
the hypothesis that they may have experienced an altered brain organization. In addition, we also found a brain pattern in adolescent transgirls which was different from that of cisgender girls and cisgender boys. This finding supports the other hypothesis, at least for adolescent transgirls, that supposes that subjective experiences can also be related to neurobiological alterations in transgender individuals.

PART 2 – EFFECTS OF TRANSGENDER HORMONE THERAPY

Part 2.1 – Pituitary Hormones (Prolactin and TSH)

Prolactin levels are lower in men than in premenopausal women, suggesting that sex steroids play a role in the secretion of this pituitary hormone [15]. As a result, for establishing hyperprolactinemia, a different upper range is used for men than for women. Measuring prolactin levels is usually the first step in the diagnostics for a prolactin-producing pituitary adenoma (prolactinoma). This is followed by pituitary imaging in the case of hyperprolactinemia. In contrast to prolactin, sex-specific reference ranges are mostly not used for the pituitary hormone thyroid-stimulating hormone (TSH). However, TSH secretion is probably somewhat higher in women than in men [16] which may be the result of inhibitory effects of androgens [17] or stimulatory effects of estrogens [18]. Some studies even recommend to use gender-specific (and age- and ethnicity-specific) TSH reference intervals to decrease misclassification of patients with thyroid dysfunction [19]. In chapter 6 and chapter 7 we examined the effect of estrogen ± cyproterone acetate therapy on prolactin levels in transwomen (chapter 6 and chapter 7) and of testosterone therapy in transmen (chapter 6).

In line with other studies [20–24] we found a large increase in the (geometric) mean prolactin level in transwomen using estrogens combined with the progestogenic cyproterone acetate, with many transwomen developing prolactin levels above the upper female reference interval. However, in postoperative transwomen using estrogens without cyproterone acetate we found that the (geometric) mean prolactin level was (almost) comparable to baseline, and no or only
few transwomen had prolactin levels above the upper female reference interval. In transmen using testosterone we found a decrease in prolactin levels. In chapter 8 we examined whether reference intervals of the experienced gender can be used for interpreting laboratory values in postoperative transgender individuals on hormone therapy. Several laboratory parameters were analyzed in this study, including prolactin and TSH. While chapter 6 and chapter 7 showed that (geometric) mean prolactin levels in postoperative transwomen were close to baseline levels, in chapter 8 we found that it is more appropriate to use female intervals than male intervals for interpreting prolactin values in postoperative transwomen receiving estrogen therapy. This can probably be explained by the right skewed distribution of prolactin levels. As expected, we found that male intervals are most appropriate to use for interpreting prolactin levels in postoperative transmen receiving testosterone therapy. For TSH we found that the upper reference range was higher in postoperative transmen than in postoperative transwomen which may suggest an effect of transgender hormone therapy on TSH secretion. However, for both postoperative transwomen and postoperative transmen it seemed still adequate to use non-sex-specific reference intervals.

In summary, we found support for effects of transgender hormone therapy on pituitary prolactin and possibly also TSH secretion. Increased prolactin levels (above the female upper reference interval) in preoperative transwomen on cyproterone acetate and estrogen therapy seems quite normal. These levels are likely to decrease after gonadal surgery/cyproterone acetate discontinuation. In the absence of prolactinoma-associated symptoms, watchful waiting may be a good alternative for pituitary imaging in case of hyperprolactinemia in this group. For postoperative transwomen and transmen it seems most adequate to use the upper reference interval of the experienced sex for diagnosing hyperprolactinemia. Although there might be an effect of transgender hormone therapy on TSH secretion, it does not have clinical consequences for the reference intervals.
Part 2.2 – The Risk Of Developing Sex Steroid-Sensitive Brain Diseases

Benign brain tumors

Sex differences have been reported for some benign brain tumors. Especially, meningiomas and prolactinomas occur more often in fertile women than in men [25–27]. This sex difference may be (partly) explained by the different sex steroid milieu between men and women, as both meningioma cells [28] and several types of pituitary cells (including lactotrophs), contain sex steroid receptors [29]. If exogenous sex steroids indeed play a role in the development of benign brain tumors, it may be worthwhile to consider alternation or discontinuation of hormone therapy in individuals who suffer from such a tumor and are receiving hormone therapy. Interestingly, a recent study suggests that progestogen-only contraception, in contrast to estrogen-only or a combination of estrogen and progesterone contraception, is associated with an increased risk of meningioma [30]. In contrast, the occurrence of prolactinomas seems not associated with the usage of contraceptives [31]. In chapter 9 we compared the occurrence of the most common types of benign brain tumors (meningioma, non-secretive pituitary adenoma, prolactinoma, somatotrophinoma, corticotrophinoma and thyrotrophinoma [32]) in transgender individuals on hormone therapy with cisgender men and women. We found that the incidence of meningiomas in transwomen was four times higher compared to cisgender women and 12 times higher compared to cisgender men. We also found that the occurrence of prolactinomas occurred four times more often in transwomen compared cisgender women, and 27 times more often compared to cisgender men. However, prolactin levels in the transwomen were regularly screened as recommended by the endocrine guidelines [33]. When we only included the symptomatic cases we found that the occurrence of symptomatic prolactinomas was 15 times higher in transwomen compared to cisgender men, but similar to that of cisgender women. These results suggest that (exogenous) sex steroids probably play a role in the occurrence of
meningiomas and prolactinomas. While the study design did not allow us to examine whether estrogen or the progestogenic anti-androgen cyproterone acetate may be responsible for the increased risk, it is noteworthy, that most transwomen had received orchiectomy but still used cyproterone acetate at time of diagnosis (cyproterone acetate is usually discontinued after orchiectomy). This may suggest that (anti-androgenic) progestogens may play a more important role than estrogens in the development of meningiomas and prolactinomas. Supporting this hypothesis, several studies have found spontaneous regression of meningiomas after discontinuation of progesterone (agonists) [34–36]. In addition, in chapter 5 and chapter 6 we have shown that prolactin levels in transwomen decrease after discontinuation of cyproterone acetate. In transmen we found two cases of acromegaly, which was unexpectedly high based on the incidences reported in cisgender men and cisgender women. The two cases observed by our study group could be added to another recently reported case of a somatotrophinoma in a transman [37]. Hypothetically, there could exist a relationship between testosterone therapy in transmen and somatotrophinomas. To date, nothing has been established about a relationship between testosterone therapy and (growth of) somatotrophinomas. It remains difficult to draw conclusions about such relationship solely based on the three known somatotrophinoma cases in transmen.

In summary, we found indications that hormone therapy increases the risk of meningiomas and prolactinomas in transwomen, and the risk of somatotrophinomas in transmen. While the risk of these types of tumors was increased, the incidence remained rare. Therefore, regularly screening the transgender population for these types of tumors seems unnecessary. However, physicians should keep in mind that there may be a relationship between hormone therapy and the occurrence of these tumors, and should consider to alter, cease, or lower the dose of hormone therapy in patients with these types of tumors.
Strokes

Observational studies have found that premenopausal women have lower risk of (ischemic) strokes than men with a similar age. It has been hypothesized that female sex steroids (estrogen and in a lesser extend progesterone) play a protective role in the development of strokes [38]. It is also possible that testosterone have a deleterious effect on one’s risk of stroke development. If sex steroids are indeed (completely) responsible for the sex difference in stroke risk, hormone therapy would hypothetically alter the risk of strokes in transgender individuals. In chapter 10 we compared the occurrence of strokes (ischemic or hemorrhagic, transient ischemic attack, or subarachnoid hemorrhage) in transgender individuals on hormone therapy with the occurrence of strokes in cisgender men and women. We found that transwomen had about a twofold higher risk of strokes compared to cisgender men and cisgender women. While not significant, which may be caused by the smaller group and shorter follow-up duration, transmen receiving testosterone, tended also to have a higher risk of strokes compared to cisgender men and cisgender women. Another recent study from 2018 supported our findings [39]. Our results do suggest that estrogen (± antiandrogen) therapy, and possibly also testosterone therapy, increases one’s risk of strokes. Both estrogen (± antiandrogen) and testosterone therapy in transgender individuals have been associated with (negative) effects on the lipid spectrum, insulin resistance, and/or hemostasis parameters [40–42]. The increased stroke risk of transgender individuals on hormone therapy may also be partly explained by psychosocial stressors and a more unhealthy lifestyle such as a higher smoking prevalence.

In summary, we found indications that transgender hormone therapy possibly increases the risk of strokes in transwomen. It is important that physicians are aware of this (potential) increased risk and try to reduce this risk by giving lifestyle advices, and by
regularly monitoring and managing cardiovascular risk factors such as lipid spectrum, glucose levels, and blood pressure.

**STRENGTHS AND LIMITATIONS**

The main strength of the studies described in **part 1** of this thesis is the uniqueness of the data. Before the start of this thesis there were almost no data available about resting-state network functional connectivity in adult transgender individuals. In addition, we were and are the only center in the world that analyzed brain MRI data (including resting-state data) of transgender children and adolescents. Limitations of the studies described in **part 1** of this thesis were the small sample sizes, the baseline usage of gonadotropin-releasing hormone analogues by the adult and adolescent transgender groups, and the inability to associate the degree of gender dysphoria to the neurobiological characteristics.

Strengths of the chapters of **part 2.1** of this thesis were, again, the uniqueness of the data. Before the start of this thesis there were no studies focusing on prolactin and TSH (reference) levels that differentiated between preoperative and postoperative transgender individuals. The main limitation of the chapters in **part 2.1** of this thesis were the inability of the study designs to fully differentiate between cyproterone acetate and estrogen effects on prolactin levels (**chapter 5** and **chapter 6**), and to examine the effect of different hormone administration routes on reference intervals (**chapter 7**).

The main strength of the chapters of **part 2.2** of this thesis were the unique large size and follow-up duration of the study population. Worldwide, there are no large transgender cohort studies with such a long follow-up duration. Despite this great strength, important limitations were the inability to compose adequate control groups to control for confounders, and the inability to differentiate among various hormone regimens.
OVERALL CONCLUSION AND FUTURE DIRECTIONS

The findings in part 1 of this thesis provide support for the neurobiological hypothesis that transgender individuals have experienced a less sex-typical/sex-atypical brain organization, but also for the hypothesis that supposes that subjective experiences may be related to neurobiological alterations in transgender individuals. These observations suggest that there may be no single neurobiological explanation for gender dysphoria but that several factors may play a role. Our findings can be used to inform transgender individuals about possible neurobiological explanations of gender dysphoria.

For future studies it would be worthwhile to confirm our hypothesis that there may be no single neurobiological underpinning for gender dysphoria and that they further unravel the association between brain characteristics and feelings of gender dysphoria.

The chapters in part 2 of this thesis provide indications for effects of transgender hormone therapy on pituitary prolactin and possibly also TSH secretion (part 2.1), on the risk of developing a benign brain tumor, and on the risk of developing a stroke (part 2.2). These findings suggest that (long-term) hormone therapy probably have wide effects on the human brain. The findings in especially part 2.2 of this thesis, suggest that long-term transgender hormone therapy may not be without risks. However, the absolute risks for brain diseases still seems low. We think that for most transgender individuals the benefits of hormone therapy outweigh the potential hormone-induced brain risks. We can use our findings to minimalize the potential negative effects of hormone therapy on the brain as much as possible, and to protect transgender individuals from over- but also underdiagnosis.

For future studies it would be worthwhile to try to differentiate between the effects of transgender hormone therapy and the effect of confounders on the risk of brain diseases in transgender individuals. In addition, it would be interesting to unravel the potential mechanism how transgender hormone therapy affect pituitary hormone (prolactin) secretion
and one’s risk of developing a benign brain tumor or stroke. Finally, since we have only focused on some brain effects of transgender hormone therapy, many of the long-term effects of transgender hormone therapy on the brain (but also on other parts of the body) are still unknown and need to be explored by future studies.
REFERENCES


