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Gender-affirming hormone treatment – a unique approach to study the effects of sex hormones on brain structure and function

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Abstract

Investigating the effects of the gender-affirming hormone treatment of transgender people using neuroimaging provides a unique opportunity to study the impact of high dosages of sex hormones on human brain structure and function. This line of research is of relevance from a basic neuroscientific as well as from a psychiatric viewpoint. Prevalence rates, etiopathology, and disease course of many psychiatric disorders exhibit sex differences which are linked to differences in sex hormone levels. Here, we review recent neuroimaging studies from others and our group that investigate the effects of gender-affirming hormone treatment in a longitudinal design utilizing structural and functional magnetic resonance imaging and positron emission tomography. Studies point to a general anabolic and anticatabolic effect of testosterone on grey and white matter structure, whereas estradiol and antiandrogen treatment seems to have partly opposite effects. Moreover, preliminary research indicates that gender-affirming hormone treatment influences serotonergic neurotransmission, a finding that is especially interesting for psychiatry. A clear picture of a hormonal influence on brain activity has yet to emerge. In conclusion, the available evidence reviewed here clearly indicates that sex hormone applications influence brain structure and function in the adult human brain.

Keywords: sex hormones, neuroimaging, transgender, gender dysphoria

1. Introduction

The rationale for studying the effect of sex hormones on brain and behavior may be driven by a general psychological and neuroscientific, as well as by a psychiatric and neuropathological interest. On the one hand, most researchers studying sex hormones from a psychological or basic neuroscientific perspective contemplate psychopathological implications in the discussion of their manuscripts. On the other hand, psychiatrists probing the impact of sex hormones on the level of symptoms establish their argument based on basic neuroscientific evidence. Meanwhile, it is common knowledge for researchers in the field that mood disorders such as major depression are twofold more prevalent in women than men, e.g., see Alonso et al. (2004). Several lines of evidence indicate that this sex difference is partly due to the organizing effects of sex hormones during critical windows of early perinatal development (Marrocco and McEwen 2016). Especially estrogen exerts significant effects on the developing brain, including on apoptosis, cell survival, neurite growth, and synaptic connectivity (McCarthy 2008). However, there is still controversy about the specific organizing role of estradiol, testosterone, and estrogenic metabolites of testosterone in the human brain and psychosexual differentiation (Bakker and Baum 2008, Baum and Bakker 2017).

In addition to these generally permanent effects, sex hormones can also exert activational effects on brain structure and function during adulthood. These effects are considered non-permanent in nature but can also result in structural, functional, as well as neurochemical changes that may be measured using in vivo neuroimaging methods in humans, such as functional and structural magnetic resonance imaging (MRI) or positron emission tomography (PET) (e.g., see Jovanovic et al. 2009, Syan et al. 2017, Pletzer et al. 2018). Such effects have their behavioral, cognitive and emotional correlate, as for example, evidenced by the known changes in mood across the menstrual cycle. Moreover, several syndromes and disorders are closely linked to increases or decreases in circulating sex hormones, such as premenstrual dysphoric disorder (PDD), postpartum depression, or depression during perimenopause. Here, the relationship between symptoms and altered sex hormone levels has been proposed to reflect a U-shaped function (Mueller et al. 2014). In contrast, other research indicates that especially changes, rather than absolute stable levels of sex hormones seem to trigger psychiatric symptoms (Schmidt et al. 2017). Finally, several lines of evidence also indicate that genetic sex and sex hormones modulate the effects of psychopharmacological treatments for psychiatric disorders (Sramek et al. 2016, Williams and Trainor 2018, Herzog et al. 2019).

Sex hormones modulate neural structure and activity on virtually all levels of cell function. This includes gene transcription, epigenetic modification of transcription, translation, as well as various intracellular signaling pathways (Rubinow and Schmidt 2019). Sex hormones thereby affect the structure and function of single cells, as well as entire cell circuits. By acting directly on ion channels, activating gene transcription factors, or modulating neurotransmitters, sex hormones change the neuro-functional basis of virtually all our mental faculties. Hence, sex hormones' role, either as causal factors, mediators or moderators, is indicated in various psychiatric disorders, including addiction (Lenz et al. 2012), autism (Baron-Cohen et al. 2015), anxiety and trauma (Li and Graham 2017), depression (Slavich and Sacher 2019), eating disorders (Hirschberg 2012), gender dysphoria (Bao and Swaab 2011) or schizophrenia (Owens et al. 2018).

Studying the effects of sex hormones on brain and behavior in humans in vivo can be done in multiple ways. A simple method is to measure hormone levels, for example in saliva or peripheral blood, and associating them with the brain signals obtained by MRI or PET in a cross-sectional or longitudinal approach. This method, as applied by others and us (e.g., Fernandez et al. 2003, Lanzenberger et al. 2011, Stein et al. 2014) has the advantage of increased feasibility compared to interventional studies but is limited in terms of interpretability given its correlational nature. In order to inform about the causal role of sex hormones, researchers may employ an interventional approach by administering single dosages of hormones and studying their effects in healthy subjects (e.g., van Wingen et al. 2008, Finkelstein et al. 2013). Here, ethical reasons limit the investigation of higher dosages or prolonged and repeated exposure. To investigate high dosages and longer-term administration of sex hormones, researchers may turn to clinical intervention approaches by probing hormonal treatments, for example in the case of Klinefelter syndrome e.g., Foland-Ross et al. (2019), postmenopausal women, e.g., Kranz et al. (2014) or transgender individuals undergoing gender-affirming hormone treatment (GHT). Especially the latter represents a unique approach to disentangle genetic sex, gender identity, and the impact of endocrine feminizing treatment in transfemale (TF) i.e., in assigned men at birth with female gender identity and masculinizing treatment in transmen (TM), i.e., in assigned women at birth with male gender identity.

The aim of the current review is to summarize recent research employing GHT as an experimental model to study the effect of sex hormones on the human brain in a longitudinal design. After an introductory chapter on GHT for transgender persons, we review recent neuroimaging research employing MRI and PET from our lab and others to study human brain structure and function in response to GHT. For cross-sectional research and a better understanding of the neurobiology of

transgenderism and its implications for GHT, the reader is referred to previous reviews (Smith et al. 2015, Guillamon et al. 2016, Mueller et al. 2017, Nguyen et al. 2019).

2. GHT for transgender persons

Gender dysphoria (DSM-5) and gender incongruence of adolescence or adulthood (ICD-11) are terms that refer to persons who identify with a gender that is different from their assigned sex at birth. Those whose gender identity is opposite to their birth assigned sex often define themselves as transgender and typically desire a transition towards the experienced gender through GHT and surgery. Prevalence rates of transgenderism vary between studies with overall meta-prevalence estimates of 6.8 (95% CI = 4.6-9.1) per 100,000 population for transgender-related diagnoses (Collin et al. 2016). GHT is based on estrogen, progesterone and testosterone administration and aims to bring sex hormone levels into the reference range of the desired gender. In doing so, GHT feminizes or masculinizes the body. GHT is, therefore, a central component in the process of gender transition (Levy et al. 2003, Gooren et al. 2008, Hembree et al. 2009, Unger 2016). Current guidelines for GHT are published by the Endocrine Society (Hembree et al. 2009) and the World Professional Association for Transgender Health (2011). GHT is tailored to the individual's goals and medical risk profile. TM typically receive testosterone esters, either orally, via intramuscular, or subcutaneous injection or transdermal application and, if menstruation persists, additionally either the progestins lynestrenol or desogestrel. TF typically receive estradiol via oral, parenteral, or transdermal route, and the anti-androgen spironolactone or the progestin cyproterone acetate in order to suppress testosterone secretion. In addition, TF may receive an alpha-5-reductase-inhibitor in case of extensive hair loss. Moreover, in some cases, both TM and TF may receive gonadotropin-releasing hormone (GnRH) agonists. This leads to a downregulation of GnRH receptors after a few weeks of treatment and repression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion, thereby dramatically reducing endogenous production of estradiol and testosterone.

Most physical changes induced by GHT take place over two years, whereas first visible changes can be expected to occur already within the first months after treatment commencement. GHT in TM typically leads to facial and body hair growth, changes in skin appearance (acne), a deepening of the voice, a decreased percentage of body fat, breast atrophy, vaginal atrophy, as well as clitoral enlargement. GHT in TF typically leads to breast growth, body fat redistribution, softening and decreased oiliness of the skin, decreased testicular volume, and a reduction of male pattern hair growth (Unger 2016). Psychological effects of GHT include a decrease of general

psychopathology, body uneasiness, and depressive symptoms. Here, especially breast growth in TF, and in TM an increase in BMI seems to contribute to the reduction in body uneasiness (Fisher et al. 2016). Sexual desire significantly increases in TM but declines in TF (Wierckx et al. 2014). In both groups, GHT seems to improve the quality of life of individuals (Gorin-Lazard et al. 2012). However, the extent of physical and psychological change varies considerably across individuals.

3. GHT effects on brain structure investigated with MRI

To date, nine published studies employed GHT to investigate the impact of sex hormones on human brain structure in a longitudinal design (see Table 1). Those studies looked at cortical and subcortical volume and cortical thickness, as well as at indices for white and gray matter microstructure changes. Most of them compared their GHT-related effects to test-retest measurements in cis-gender controls, that is, to persons in which gender identity and sex assigned at birth are aligned.

3.1. Testosterone treatment in TM

Five studies looked at the effects of testosterone treatment on total brain volume. Three of them comprising of 61 TM indicated increases in total brain (Hulshoff-Pol et al. 2006, Kilpatrick et al. 2019) or total gray matter volumes (Zubiaurre-Elorza et al. 2014). In contrast, two studies comprising of 47 TM showed no significant changes (Seiger et al. 2016, Burke et al. 2018). Significant increases ranged from 0.5 to 1.5% of baseline values. On the regional level, volume increases were found in the thalamus (1-3% of baseline levels) (Zubiaurre-Elorza et al. 2014, Seiger et al. 2016) and bilateral pallidum (2% of baseline levels) (Seiger et al. 2016), although studies also indicated no changes in several subcortical regions that exhibit high androgen receptor densities including the hippocampus (Seiger et al. 2016, Burke et al. 2018), amygdala, caudate nucleus, putamen, thalamus (Burke et al. 2018), and hypothalamus (Hulshoff-Pol et al. 2006). On the other hand, studies observed increases in cortical thickness (CTh) in several frontal, temporoparietal, and occipital cortex regions (2.5-3.5% of baseline levels) (Zubiaurre-Elorza et al. 2014, Seiger et al. 2016, Kilpatrick et al. 2019). However, when controlling for the change in total grey and white matter volume, Kilpatrick et al. (2019) observed CTh reductions compared to controls. Authors of the latter study interpreted this as a treatment induced change specific to self-body perception processing circuits.

To further elucidate testosterone effects on brain volume and CTh, several studies investigated the association between GHT-induced changes in testosterone levels and changes in volumetric measures. Zubiaurre-Elorza et al. (2014), for example, observed a positive correlation between testosterone changes and CTh changes in parieto-temporo-occipital regions indicating a dose-dependent volume-increasing influence on these structures. On the other hand, Seiger et al. (2016) found no significant relationship, whereas Hahn et al. (2016) observed a negative correlation between testosterone increases and volume changes in left temporal and frontal language areas, indicating a volume-reducing effect of testosterone. Hence, although several studies point towards increasing volumes and CTh upon testosterone treatment, definite region-specific conclusions must remain preliminary. Instead, a general anabolic and anticatabolic effect of testosterone on brain volume may be assumed (Guillamon et al. 2016).

So far, only one study investigated the effects of androgenization in TM on gray matter microstructure (Kranz et al. 2018). We focused our analysis on the hypothalamus, given its central role in the endocrine system, and utilized diffusion-weighted imaging to study 25 TM before, after one, and after four months into GHT. Although we did not observe overall changes in diffusivity measures compared to controls, a separate post-hoc analysis only including TM revealed significant reductions in mean diffusivity (MD) (up to 7 % change from baseline) unilaterally after one, and bilaterally after four months of testosterone treatment in the lateral hypothalamus. Moreover, increases in testosterone levels were significantly correlated with reductions in MD, pointing towards a dose-dependent hormone effect. The specific physiological process behind MD reductions remains to be elucidated. However, although preliminary, our study indicates that testosterone changes hypothalamic architecture and thereby potentially affecting male sexual motivation (Kranz et al. 2018).

Regarding the brain's white matter, four published studies looked into the effects of GHT using diffusion-weighted MRI so far (Rametti et al. 2012, Hahn et al. 2016, Kranz et al. 2017, Burke et al. 2018). All of them studied white-matter microstructure and employed a diffusion-tensor model to determine the three principal diffusivities and their derivatives MD and fractional anisotropy (FA). Comprising a total of 84 TM, studies consistently found testosterone treatment induced increases in FA, although discrepancies exist regarding the specific tracts and fasciculi. Rametti et al. (2012), for example, observed FA increases of about 5% and 8% to baseline values in the right superior longitudinal fasciculus and right corticospinal tract, respectively. Conversely, Burke et al. (2018) found increases confined to the posterior part of the right inferior fronto-occipital fasciculus, whereas we (Kranz et al. 2017) observed increases only in the left hippocampal cingulum and right occipital blade. FA increases in the latter study were correlated with changes

in estradiol plasma levels, indicating a causal effect of hormonal changes on white matter microstructure.

In addition to FA, MD was investigated in Kranz et al. (2017) and Hahn et al. (2016), two of our studies that were based upon partly the same pool of participants. We observed MD reductions in several long-range, as well as cortico-cortical tracts, especially after four months of treatment. Moreover, there was a positive correlation between estradiol changes and MD changes (Kranz et al. 2017). Looking specifically at the extreme capsule pathway that connects Broca's with Wernicke's area, whose volumes were shown to be affected by testosterone (see above), Hahn et al. (2016) observed a negative correlation between testosterone changes and MD changes. These results indicate a modulatory role of testosterone as well as estradiol on white matter microstructure, although we note that findings may also be explained by regression toward the mean. This cautionary note is based on significant differences between transgender groups and control participants before treatment commencement, as published by us in Kranz et al. (2014). In any case, even if we assume a causal role of sex hormones, the specific biological basis of hormone-induced diffusivity changes remains a matter of future studies. In the pathological context, FA and MD changes are typically related to axonal integrity and membrane damage. Conversely, in the context of healthy brains, as is the case of this review, the biological basis of water diffusion must be searched in the organizational properties of neural fibers. However, whether effects can be pinpointed to changes in axon diameter, axonal density, axonal transport or, myelin staining remains an unanswered question.

3.2. Estradiol and antiandrogen treatment in TF

Five of the above-mentioned studies also investigated the effects of GHT in TF, comprising a total of 74 participants. Overall, studies indicated volume decreases after GHT, including total brain volume (2% of baseline values) (Hulshoff-Pol et al. 2006), total grey and white matter volume (1% and 2% of baseline values, respectively) (Kilpatrick et al. 2019), as well as subcortical volumes of the hypothalamus (Hulshoff-Pol et al. 2006), thalamus and pallidum (Zubiaurre-Elorza et al. 2014) and hippocampus (Seiger et al. 2016) (decreases of 1-5% of baseline values). Moreover, total CTh seemed to decrease (Zubiaurre-Elorza et al. 2014), whereas ventricle volumes increased after GHT commencement (Hulshoff-Pol et al. 2006, Zubiaurre-Elorza et al. 2014, Seiger et al. 2016). However, treatment-related effects cannot be easily isolated to a specific hormone because GHT in TF comprises estradiol administration, as well as antiandrogenic progestogen

treatment. Nevertheless, in Seiger et al. (2016), we found a positive correlation between progesterone and hippocampus as well as caudate volume reductions, indicating a direct neuroprotective effect of progesterone. Moreover, a recent study by Schneider et al. (2019a) tested regional CTh in 18 TF who had already undergone GHT and gender-affirming surgery (surgical hypogonadism). Participants were measured for a baseline scan after a one-month wash-out phase, and again in a second scan two months after reintroduction estradiol treatment. Authors observed increases as well as decreases in regional CTh, although findings did not survive corrections for multiple comparisons. However, significant negative correlations were observed between estradiol changes and CTh changes in the left superior frontal gyrus, the left middle temporal gyrus, the right precuneus, the right superior temporal gyrus, and the right pars opercularis. These results point to a causal role of estradiol for CTh reductions.

Except for the study by Kilpatrick et al. (2019), who observed total white matter reductions as stated above, there is only one other study that investigated white matter changes in TF (Kranz et al. 2017). The authors studied the effects of GHT in 15 TF (in addition to 29 TM as discussed above) using diffusion-weighted MRI. They found MD increases in the left, and decreases in the right splenium of the corpus callosum and an increase in the right temporal blade. Moreover, GHT led to a reduction in FA in the right post-central blade. However, no correlations between these changes and hormonal changes were observed.

Taken together, studies investigating GHT in TF are limited in number but show a fair amount of congruence. They indicate that the effects of estrogen and antiandrogen treatment on brain structure in TF seem to be opposite to the effects of testosterone treatment in TM. That is, brain volumes and CTh decline in TF upon GHT, whereas white matter FA and MD increase and decline, respectively. These changes are several times the magnitude of the average age-related changes observed in cisgender adults (Inano et al. 2011, Peng et al. 2016).

4. GHT effects on brain function investigated with MRI

To date, six published studies comprising a total of 64 TF and 101 TM investigated the effects of GHT using functional MRI in a longitudinal design (see Table 1). Five of these studies probed blood-oxygen-level dependent (BOLD) response at the so-called “resting-state,” and calculated functional connectivity between regions to investigate GHT effects on functional brain networks. An exception is Sommer et al. (2008) who investigated brain activation in six TM and eight TF during language and mental rotation tasks. The authors observed a trend towards language-related BOLD reduction after GHT in the combined sample (including TF and TM). Hence, no

clear conclusion about hormone-specific effects can be drawn from this study. Moreover, results must be interpreted with caution, given the low sample size of this study. The summary of the five studies employing resting-state fMRI is again presented separately for the two treatment arms, as done in chapter 3.

4.1. Testosterone treatment in TM

Heterogeneous results are provided by a handful of studies that investigated the effects of testosterone treatment in TM. Inconsistencies are due to methodological differences, as well as the specific networks chosen for investigation. For example, in Hahn et al. (2016), which is already mentioned in section 3.1., we focused specifically on Broca's and Wernicke's area and their connection. In addition to a volume-reducing effect of testosterone in these two language areas, we observed a positive correlation between testosterone changes and changes in the functional connectivity between the two regions. We interpreted this finding as a compensatory strengthening of the connecting pathway, which was also reflected in changes of the white matter microstructure, as already described above. In a study by Spies et al. (2016), we adopted a whole-brain approach to investigate functional connectivity using network-based statistics that requires no a priori selection of regions. Investigating 33 TM before, as well as one and four months into testosterone treatment, we observed no significant changes, as well as no significant correlation between testosterone increases and network changes. This null finding concords with a study by Nota et al. (2017) who investigated functional network connectivity in 22 TM at baseline and after four months of testosterone treatment. However, baseline measurements were performed while participants were already receiving GnRH agonists for two months. Hence, estradiol levels were already suppressed at baseline in these participants.

Finally, in the study by Burke et al. (2018), which also investigated brain volumes and structural connectivities in 22 TM, as already described above, authors observed increases, as well as decreases in functional connectivity upon testosterone treatment. Using the left temporo-parietal junction as seed, authors observed increased connectivity to left temporal and frontal cortices. However, using the right temporo-parietal junction as seed, GHT led to a decreased connectivity to the left inferior frontal gyrus compared to controls. The authors interpreted these changes with respect to GHT-induced changes in the neural representation of body perception. Taken together, there seems to be no consistent effect of testosterone on functional connectivity upon GHT in TM among studies. This may be due to the methodological differences and the choice of networks

investigated. At best, one may conclude that the presumed effect of testosterone on functional networks varies depending on the specific network and its function.

4.2. Estradiol and antiandrogen treatment in TF

In Spies et al. (2016), as mentioned above, we also investigated 24 TF before and during anti-androgen and estrogen treatment. Using network-based statistics, we observed a significant increase in a network clustered around the supramarginal gyrus over the course of GHT. Although there was no correlation between hormone level changes and network changes, we observed a negative correlation between network increases and a change in psychological measures reflecting aspects of empathy. This was in contrast to our hypothesis and to previous research indicating a positive relationship between empathy and resting-state functional connectivity (Otti et al. 2010, Takeuchi et al. 2014). However, given that the supramarginal gyrus is central to interpersonal cognition and emotion, we interpreted this finding as a GHT-induced change in the network correlates of empathic processing. As in the study by Spies et al., (2016), both transgender groups were also studied in Nota et al. (2017). Authors focused on four networks, the default-mode network, salience network, and right and left working memory network. However, no significant effects of GHT were observed; neither in the 22 TM as mentioned above nor in 14 TF undergoing anti-androgen and estrogen treatment.

Finally, a recent study by Schneider et al. (2019b) tested functional connectivity in the same 18 TF as in Schneider et al. (2019a) mentioned above. Participants were measured for a baseline scan after a one-month wash-out phase, and again in a second scan two months after reintroduction estradiol treatment. Using the thalamus as seed region and voxels in the sensorimotor cortex and dorsal striatum as targets, the authors observed an increase in functional connectivity upon GHT reintroduction. In an exploratory, data-driven whole-brain approach, authors further observed a decrease in functional connectivity between the subcallosal and the medial frontal cortex. Although participants were not naïve to GHT at baseline, the authors concluded that their data indicate estradiol-mediated changes in functional networks underlying cognitive, emotional, and sensorimotor processes. Taken together, studies on the effects of GHT in TF are sparse and inconsistent. Future studies are needed to unravel the seemingly complex causal relationship between sex hormones and resting-state connectivity.

5. GHT effects on neurotransmitter systems investigated with PET

To our knowledge, only one study from our group has been conducted to investigate the effects of GHT using molecular brain imaging with PET so far (Kranz et al. 2015). We focused on the serotonergic system using the radioligand [^{11}C]DASB to quantify serotonin transporter binding, an approximation of the serotonin transporter density expressed on the cell surface. We chose the serotonin transporter (5-HTT) as a target since previous preclinical research indicated changes in serotonin uptake, 5-HTT mRNA expression, and 5-HTT binding upon estradiol and testosterone exposure (McQueen et al. 1999, Bethea et al. 2002). We included 14 TM and 19 TF and measured them before, one month, and four months after GHT commencement. We further included 35 control participants (24 cis-males, 11 cis-females) to determine the test-retest variability of our PET data. Results showed a progressive increase in 5-HTT binding from baseline to one and four months into testosterone treatment in TM. 5-HTT increases were seen in transporter-rich regions such as the midbrain, thalamus, and basal ganglia, but were also strong in amygdala and hippocampus (up to 20% increase of baseline levels). Testosterone plasma level increases were positively correlated with 5-HTT binding, substantiating our interpretation of a causal effect of testosterone on 5-HTT expression. Conversely, TF exhibited a numerical but non-significant increase in 5-HTT after one month into antiandrogen and estrogen treatment, whereas 5-HTT binding significantly decreased after four months of treatment. Interestingly, changes in 5-HTT binding were positively correlated with changes in estradiol plasma levels, indicating a protective effect of estradiol against 5-HTT expression decline.

Previous rodent research showed that testosterone but not 5α-dihydrotestosterone administration increased 5-HTT mRNA and 5-HTT density (McQueen et al. 1999). Unlike 5α-dihydrotestosterone, testosterone is likely to be aromatized to estradiol. Hence, 5-HTT binding increases upon testosterone administration in TM in our study may depend on the conversion of testosterone to estradiol. Therefore, it is not testosterone but estradiol that modulates 5-HTT gene expression via activated estrogen receptors. This interpretation also concurs with the positive correlation between 5-HTT binding changes and estradiol changes observed in TF.

Neurotransmitters are of special interest to psychiatrists because psychopharmacological treatments such as SSRIs directly target neurotransmitter function. It is important to keep in mind that the 5-HTT is usage dependent (Steiner et al. 2008). Increased levels of synaptic serotonin concentration will result in an increase in active 5-HTT expressed on the cell surface, whereas if serotonin concentrations decline, 5-HTT proteins will internalize and eventually degrade. Following this line of thinking, our results may indicate an increase in serotonergic neurotransmission upon testosterone treatment. Therefore, our results provide a biological explanation for the antidepressant effect of testosterone treatment in hypogonadal men

(Amanatkar et al. 2014). However, it remains to be clarified whether steroid hormones act directly on 5-HTT expression via their intracellular activated receptors, or whether they modulate other aspects of serotonergic neurotransmission (e.g., serotonin synthesis, receptor expressions, or serotonin degradation), and thereby affect 5-HTT expression indirectly. In any case, results from our study must be seen as preliminary, given the small sample size. More research is needed to replicate our findings, and future research will determine the exact pathways by which sex steroids modulate serotonergic neurotransmission in the human brain.

In addition to serotonin, preclinical research also points to sex hormone effects on other neurotransmitters such as dopamine (Alderson and Baum 1981, Yoest et al. 2014), norepinephrine (Wang et al. 2015), GABA (Chaudhari and Nampoothiri 2017), glutamate (Farkas et al. 2018), or endorphins (Pluchino et al. 2009). However, to the best of our knowledge, no human research has probed such effects using GHT.

6. Discussion and summary

Studies using GHT in transgender persons to investigate the effect of sex hormones on brain structure and function have provided a sizable volume of results so far. The available neuroimaging evidence indicates that sex hormone applications influence brain structure and function in the adult human brain. Moreover, longitudinal research is flanked by several cross-sectional studies that contribute to our current understanding of GHT-related effects; for structural studies, see for example Mueller et al. (2017) or Spizzirri et al. (2018), for functional studies, see (Schoning et al. 2009, Carrillo et al. 2010, Oh et al. 2012, Kim and Jeong 2014, Kim et al. 2016, Mueller et al. 2016, Clemens et al. 2017). Of note, Kim et al. (2016) showed a significant correlation between neural activation during viewing female nude pictures and free testosterone levels in postoperative TM. These results concur with our interpretation of the effects of testosterone on hypothalamic architecture (Kranz et al. 2018) and substantiate the assumed impact of GHT on sexual arousal. Moreover, cross-sectional research can provide valuable clues about the long-term effects of hormone exposure with relatively little effort compared to longitudinal analyses. Whereas longitudinal studies often only investigate GHT effects in the order of months, cross-sectional studies can easily examine transgender persons who had been on GHT for several years. For example, transgender persons who had been on GHT for around seven years were investigated by Mueller et al. (2016). The authors observed a significant negative correlation between circulating androgens and local functional connectivity in the

cerebellum. This effect was associated with treatment duration, a finding indicating activational effects of long-term administration of androgens in the adult cerebellum.

However, cross-sectional research has to be interpreted with caution because GHT-related effects are often confounded with group membership, that is, with the neural correlates of gender identity and sex assigned at birth. Indeed, several studies demonstrated structural and functional neural correlates of gender identity by comparing cis- with transgender persons before GHT commencement, for reviews, see Smith et al. (2015), Guillamon et al. (2016), Mueller et al. (2017), or Nguyen et al. (2019), for original research from our group, see Hahn et al. (2014), Kranz et al. (2014a), or Kranz et al. (2014b). Moreover, sexual orientation, as well as specific aspects related to the condition of transgender people, such as self-body perception and gender-dysphoria itself, need to be taken into account when interpreting studies on GHT in cross-sectional, but also in longitudinal designs. In other words, sexual orientation is known to have its neural correlate; hence, androphilia and gynephilia need to be considered in research on transgender GHT (Guillamon et al. 2016). Similarly, transgender brains may be different from cis-gender brains regarding their structural and functional organization as well as how they are modulated by, or interact with, sex hormones. Therefore, any general conclusion about the effects of sex hormones on adult brain structure and function must be made with caution. In addition, GHT is individually adjusted based on desired effects and side-effects, leading to substantial variability in observed results. This may be aggravated by differences in the absorption and metabolism of transgender individuals. Despite these cautionary notes, GHT is one of few ways to probe the effects of high-dosages of sex hormones in the adult human brain. The available volumetric data presented here point to a general anabolic and anticatabolic effect of testosterone on brain volumes. Also, white matter seems to be affected in this way. Conversely, estradiol and antiandrogen treatment seems to reduce brain volumes while a clear picture for white matter microstructure has yet to emerge. Similarly, revealing a consistent picture of the effects of GHT on brain activity remains a matter for future research.

Finally, the discrepancy of some of the findings presented here may be explained by the limited sensitivity of neuroimaging methods, compared to histochemical and other laboratory research techniques. Indeed, strong and consistent effects of sex hormone manipulations on hypothalamic volume has been observed in animal studies (e.g., Park et al. 1997). Studies investigating postmortem human brains (e.g., Zhou et al. 1995, Taziaux et al. 2012), also provide more definite answers compared to any of the *in vivo* imaging studies reviewed here.

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8. Author contributions

R Lanzenberger and GS Kranz designed the original research from our group, GS Kranz, P Handschuh and V Ritter curated the data, P Handschuh and V Ritter managed project administration, R Lanzenberger and GS Kranz secured the funding, GS Kranz and BBB Zhang performed the literature search and wrote the original draft of the review. All authors reviewed and edited the review and approved the final manuscript.

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Table 1: GHT effects on brain structure and function, investigated with structural and functional MRI

Author	Sample	#months of GHT	Imaging details	Measures	Effect of GHT	
					TM	TF
Burke et al., 2018	22 TM, 12 FC, 7 CM	14	3T Scanner, Volumetrics, DTI, Resting-state fMRI	Vertex-wise CTh, Subcortical ROIs, Voxel-wise FA in WM, Functional connectivity using TPJ and pAAC as seed regions	↑ CTh in several regions, ↑ FA in a posterior part of right inferior fronto-occipital fasciculus ↑ functional connectivity between TPJ and mPFC ↓ functional connectivity between TPJ and IFG	Not investigated
Hahn et al., 2016	18 TM 16 CF	1	3T Scanner, Volumetrics, DTI, Resting-state fMRI	Voxel-based morphometry, Tractography of arcuate fasciculus and extreme capsule pathway, functional connectivity between Broca's and Wernicke's areas, Correlation with hormone changes	Negative correlation between testosterone changes and 1) volume changes in Broca's and Wernicke's area, 2) MD in the extreme capsule pathway, Positive correlation between testosterone changes and functional connectivity changes between Broca's and Wernicke's areas	Not investigated
Hulshoff-Pol et. al., 2006	6 TM, 8 TF	4	1.5T Scanner, Volumetrics	Total brain volume, Hypothalamus volume, Ventricle volume	↑ Total brain volume	↓ Total brain volume, ↓ Hypothalamus volume, ↑ Ventricle volume
Kilpatrick et al. 2019	40 TM, 24 TF 11 CF 8 CM	>6	3T Scanner, Volumetrics	Total grey and white matter volume, Vertex-wise CTh	↑ Total white matter volume, ↑ CTh in insular and temporal cortex, ↓ CTh in prefrontal and parietal cortex when corrected for GM and WM volume	↓ Total grey and white matter volume, ↓ CTh in several regions, ↓ CTh in prefrontal and parietal cortex when corrected for GM and WM volume
Kranz et al., 2018	25 TM 12 CF 13 CM	1 & 4	3T Scanner, DWI	Voxel-wise MD in a dienzephalic volume covering the hypothalamus	↓ MD in the right (1 month) and bilateral (4 months of GHT) lateral hypothalamus, Negative correlation between testosterone changes and MD changes	Not investigated

Kranz et al., 2017	29 TM, 15 TF 18 CF 15 CM	1 & 4	3T Scanner, DTI	Voxel-wise MD and FA in WM, Correlation with hormone changes	↓ MD in several tracts, ↑ FA left hippocampal cingulum and right occipital blade, Positive correlation between estradiol changes and MD changes in several tracts after 1 month of GHT and negative correlation between estradiol and FA changes in several tracts after 4 months of GHT	↑ MD in left splenium of corpus callosum and right temporal blade, ↓ MD in right splenium of corpus callosum, ↓ FA in right post-central blade
Nota et al., 2016	22 TM, 14 TF 20 CF 17 CM	4	3T Scanner, Resting-state fMRI	Functional connectivity within the default-mode network, salience network, and right and left working memory network	No significant results	No significant results
Rametti et al., 2012	15 TM	>7	3T Scanner, DTI	Voxel-wise FA in WM, Correlation with testosterone at baseline	↑ FA in right superior longitudinal fasciculus and right corticospinal tract, Positive correlation between testosterone at baseline and FA changes	Not investigated
Schneider et al., 2019a	18 TF	2	3T Scanner, Volumetrics	Vertex-wise CTh, Correlation with estradiol changes	Not investigated	↑ CTh in left precentral gyrus and right precuneus (uncorrected p) ↓ CTh in right right lateral occipital cortex (uncorrected p), Negative correlation between estradiol changes and CTh changes in left SFG, left MTG, right precuneus, right STG and right pars opercularis
Schneider et al., 2019b	18 TF	2	3T Scanner, Resting-state fMRI	Functional connectivity between thalamus and sensorimotor cortex and striatum, as well as exploratory whole-	Not investigated	↑ functional connectivity between thalamus and sensorimotor cortex/putamen, ↓ functional connectivity between subcallosal cortex and medial frontal cortex

				brain analysis, Correlation with estradiol changes		
Seiger et al., 2016	25 TM, 14 TF 14 CF 12 CM	4	3T Scanner, Volumetrics	Vertex-wise CTh, Subcortical VOIs, Ventricle volume, Correlation with hormones	↑ CTh globally, ↑ volumes of left thalamus and bilateral pallidum, ↓ Ventricle volume (results observed only at an uncorrected p-threshold)	↓ CTh globally (uncorrected p), ↓ Volumes of bilateral hippocampus, amygdala and right caudate and putamen, ↑ Ventricle volume, Positive correlation between progesterone changes and right hippocampus and caudate volume changes, as well as between testosterone changes and right amygdala volume changes
Sommer et al., 2008	6 TM, 8 TF	3	1.5T Scanner, fMRI	BOLD response to two language tasks (paced verb generation task with covert articulation, paced categorical decision task) and a mental rotation task, Laterality index, Correlation with hormones	# Trend for ↓ in number of activated voxels in a priori VOIs during a language task, Positive correlation between post-GHT estradiol and language activation after treatment, Positive correlation between post-GHT testosterone and mental rotation-related activation after treatment	# effects observed in the entire sample (including TM and TW); no separate analysis performed
Spies et al., 2016	33 TM, 24 TF, 44 CF 33 CM	1 & 4	7T Scanner, Resting-state fMRI	Functional connectivity using network-based statistics Correlation with hormones and empathy scores (BVAQ, ECS)	No significant results	↑ functional connectivity of a network clustered around SMG Positive and negative correlation between SMG connectivity changes and changes in BVAQ and ECS scores, respectively
Zubiaurre-Elzora et al., 2014	15 TM, 14 TF	>6	3T Scanner, Volumetrics	Total brain volume CTh in cortical ROIs, Subcortical VOIs, Ventricle volume, Correlation with hormone changes	↑ Total grey matter volume, ↑ right Thalamus volume, ↑ CTh in several regions, Positive correlation between testosterone changes and	↓ total gray matter, ↓ right Thalamus volume, ↓ right Pallidum volume, ↓ CTh in several regions, ↑ ventricle volume

					CTh changes in several regions	

Abbreviations: ↑ represents an increase, ↓ represents a decrease compared to baseline; BOLD, blood oxygen level dependent; BVAQ, Bermond-Vost Alexithymia Questionnaire; CF, cis-female controls; CM, cis-male controls; CTh, cortical thickness; DTI, diffusion-tensor imaging; DWI, diffusion-weighted imaging; ECS, Emotional Contagion Scale; FA, fractional anisotropy; GHT, gender-affirming hormone therapy; GM, grey matter; IFG, inferior frontal gyrus; ICV, intra-cranial volume; MD, mean diffusivity; mPFC, medial prefrontal cortex; MTG, middle temporal gyrus; pAAC, pregenual anterior cingulate cortex; ROI, region of interest; SFT, superior frontal gyrus; SMG, supramarginal gyrus; STG, superior temporal gyrus; TF, trans-female; TM, trans-male; TPJ, temporo-parietal junction; VOI, volume of interest; WM, white matter.