university of groningen

How is the brain affected from gender-affirming hormone treatment?

Essay

Afroditi Tzepapadaki
S4503600

Supervisor: Bauke Buwalda

November 2021

ABSTRACT

Transgender individuals experience an incongruence between their gender identity and sex assigned at birth. When this incongruence is a cause for significant distress, it is characterized as gender dysphoria in the DSM-5. During the most recent years, gender dysphoria (GD) is gaining scientific interest due to the steadily increasing amount of referrals of transgender people experiencing GD symptoms. Clinically, patients with GD are treated with gender-affirming hormone treatment to enhance the biological sex characteristics of their identified gender. However, very little is known about the effects of this treatment on the brain of individuals with GD nor the neurobiological mechanisms behind GD. Up to recently, research was associating GD with altered cerebral sex dimorphism disregarding the main feature of GD, which is the strong perception of incongruence between one's sense of self and one's body. This essay aims to investigate the effect of gender-affirming hormone treatment on the brain from a structural and functional perspective with particular interest in neuronal circuit activation and connectivity mediating the self ("self-referential") and own-body perception processes in an effort to gain further insight into the mechanisms underlying GD.

TABLE OF CONTENTS

1. INTRODUCTION

1.1 GENDER IDENTITY

During the most recent years, the societal understanding of gender identity, gender expression and diversity has evolved, opening doors for research in the field of transgender health. An increasing amount of people view gender as a continuum with the typical male/female forming the binary identities for all the identities on the spectrum. Gender identity is one's fundamental, inner sense of their experienced gender, which can be subjected to change over the course of their lifespan (Khorashad et al., 2021). The term transgender or trans refers to individuals with gender incongruence (GI); whose gender identity does not align with their gender and sex assigned at birth. More accurately, transgender is an umbrella term utilized for binary and non-binary trans people depending on whether they identify as female/male or another -if any- gender, accordingly (Jones et al., 2019). For the purpose of this essay, the term transgender will be utilized to describe binary trans people, whereas non-binary trans individuals will be specifically mentioned as such, due to the higher amount of published papers on the former subgroup.

1.2 GENDER DYSPHORIA

Based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), gender dysphoria (GD) is classified as a significant distress or impairment in function due to the incongruence of someone's experienced and assigned gender for at least 6 months (American Psychiatric Association, 2013). GD is defined separately for children (302.6), adolescents (302.85) and adults (302.6) followed by different assessment procedures (Claahsen - van der Grinten et al., 2021). Individuals suffering from gender dysphoria are highly likely to experience body dysphoria as well as body-related avoidance by trying not to look at mirrors or hide their body under baggy clothing due to the fact that their own body perception and/or body sex phenotype do not correspond to their sense of self (Cohen-Kettenis & Pfäfflin, 2010). Since the perception of one's body is influenced by the dynamic balance between the perception of one's physical appearance based on self-observation or the reactions of others (Cash and Pruzinsky 2004) and one's body representation in the brain (Vocks et al. 2010), research has been focusing on the neuronal networks behind higher order social cognition, self-other distinction, and (own) body representation (Burke et al., 2018, 2019).

In order to meet the needs of individuals and especially youth, with GI/GD, a multidisciplinary team of care professionals is required to guide the patient and their family during the whole process. Typically, a phased trajectory is formulated by mental health professionals, psychiatrists, endocrinologists, gynecologists, surgeons, and other healthcare providers, starting with psychological assessment, followed by medical interventions such as endocrine treatment and surgical procedures (Fig. 1). Of note, many individuals with GD elect not to proceed with surgical alterations. Every step of the treatment plan is to be monitored by the multidisciplinary healthcare team in combination with continued psychological support. Depending on the age of the patient, the endocrine intervention would consist of two phases; first the pubertal suspension at the minimum Tanner stage 2 (12 years old, on average) (see 1.3), which is fully reversible, followed by irreversible treatment with gender affirming hormones, when the patient reaches the age of consent (typically at 16 years old) (Fig 1) (Claahsen - van der Grinten et al., 2020; (*Standards of Care V7*, 2011). Commencing treatment at an early age could be beneficial, as the outcome on social life and mental health is reported as largely positive (Leinung et al., 2013). During the recent years, a remarkable increase in the number of children and adolescents with GI and GD seeking professional support has been reported and interestingly, a shift in the

sex ratio of referred adolescents has been noted, with a prevalence of birth assigned females (AFAB) than birth assigned males (AMAB) (NM Graaf, 2018). In addition, it has been observed that children with GI do not necessarily show symptoms of GD, hence their support system is required to incorporate waiting steps in the plan of gender affirming interventions (J Ristori, 2016).

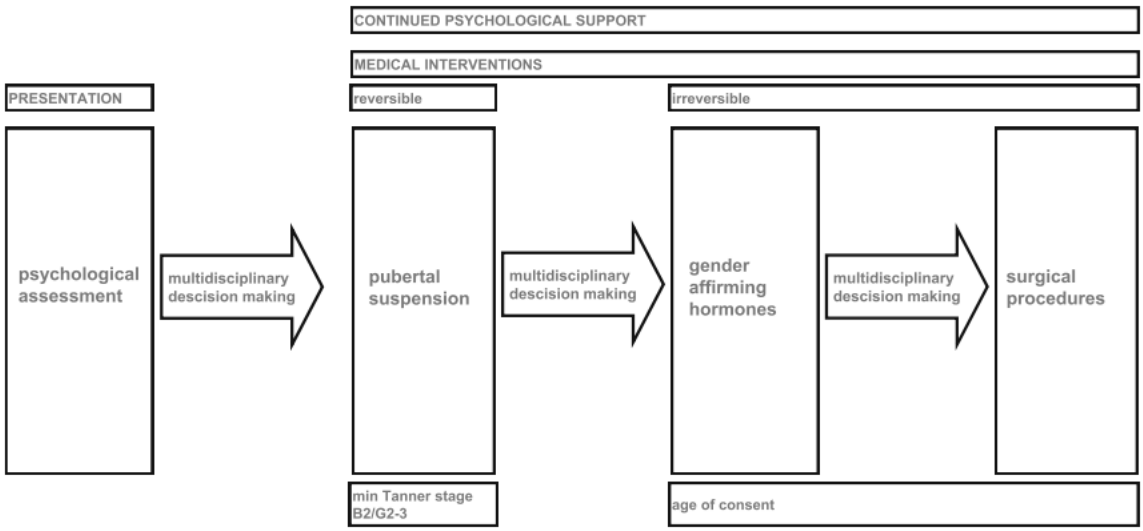

Figure 1. Approach towards children and adolescents with GI/GD (Claahsen - van der Grinten et al., 2020).

1.3 PUBERTY SUPPRESSORS

During puberty, the development of the biological secondary sex characteristics is a cause of distress for individuals with GI/GD. The physical changes of pubertal development are under the control of the hypothalamic-pituitary-gonadal axis; the gonadotropin-releasing hormone (GnRH), binds to the GnRH receptor (GnRHR) at the hypothalamus, triggering the release of the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary, which in turn regulate gonad function and sex steroid synthesis (Fig 2).

In order to suppress gonadotropin release and effectively regress or cease the progression of puberty, GnRH, GnRH analogues (GnRHa) or GnRH antagonists are administered to adolescents. In most cases, the adolescents receive an intramuscular injection every 3 months of GnRH or long lasting GnRH analogues, with GnRHa being the preferred course of treatment with established effectiveness in treating central precocious puberty (Hembree, Cohen-Kettenis, Delemarre-Van De Waal, et al., 2009; Roth et al., 2005). GnRHa, such as leuprorelin or triptorelin, are long-acting agonists of GnRH, binding to GnRHR and their administration causes an initial “flare up” response of the pituitary-gonadal axis, which soon subsidizes, the continued stimulation of the receptor, creating a negative feedback loop on the pituitary gland, leading to the desensitization of the GnRH receptor and reduction of gonadotropin secretion (Roth et al., 2005). In contrast, GnRH antagonists act by competitive binding to the pituitary GnRHR, preventing the action of endogenous GnRH, immediately suppressing pituitary gonadotropin secretion offering a more direct and dose-dependent treatment alternative. However, the antagonists treatment has a short-term effect requiring an injection every 3 days, which is unattainable over such a long period of time and there are no clinical data confirming their superiority in effectiveness in humans compared to GnRHa (Roth et al., 2005; WC Hembree, 2017). GnRHa are able to completely inhibit LH secretion and therefore gonadal testosterone production, thereby reducing circulating testosterone levels by 95% or into the castrate/female range in

AMABs. AFABs treated with GnRH analogues, experience the inhibition of estrogens and progesterone production. Due to the GnRHa high cost, both may be treated with progestins, such as medroxyprogesterone, to suppress gonadotropin secretion leading to a mild peripheral antiandrogen effect in AMABs as well as suppression of ovulation and progesterone production for long periods of time in AFABs, although residual estrogen levels may vary (Roth et al., 2005). However, this is a less effective alternative and the long-term administration of progestins may entail the risk of breast cancer, adrenal dysfunction and bone growth impairment (Roth et al., 2005).

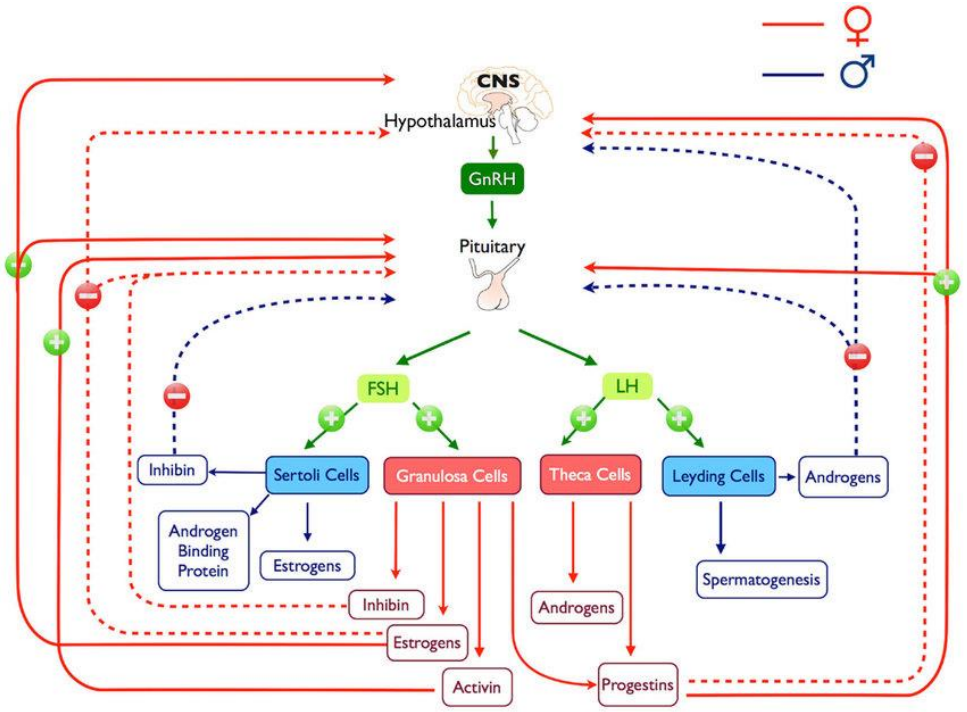

Figure 2. Representation of the hypothalamus-pituitary-gonadal axis. Positive and negative feedback loops for AFABs and AMABs. Positive feedback depicted with continuous and negative with fragmented arrows. Red for AFABs and blue for AMABs. GnRH released from the hypothalamus regulates the FSH and LH gonadotropin synthesis and secretion from the anterior pituitary. FSH and LH control steroidogenesis (estradiol, progesterone and testosterone synthesis), gametogenesis and ovulation (Durán-Pastén & Fiordelisio, 2013).

Currently, the developmental Tanner stage 2 is obligatory in order to begin puberty suppression; at this stage adolescents have experienced the onset of puberty (for birth assigned females breast Tanner 2 and for birth assigned males testicular volume of 6-8ml). Most studies following this approach included participants at least 12 years old, even though some children may reach Tanner stage 2 at the early age of 9, as well, which is confirmed with diagnostic hormone level tests.

This course of treatment has a dual purpose. First, to offer the opportunity to adolescents to explore their gender identity and changes of their pubertal development without any permanent alterations, as days after the cessation of the treatment, sex typical gonad function is restored (Hembree et al., 2017). Within this timeframe, diagnostics and mental health evaluations are conducted by the patient's care team. Secondly, the puberty suppressors prevent or slow down the development of their biological sex characteristics, such as breast size and testes volume, hence alleviating the symptoms of GD and facilitating their transition as the sex characteristics are not clearly defined in case they want to pursue with surgical

interventions (Claahsen - van der Grinten et al., 2020). Pubertal suppression does not necessarily lead to sex reassignment or social transition, as some individuals solely opt to discontinue the treatment after a few years, whereas others proceed with the gender-affirming hormone regimen (*Standards of Care V7*, 2011). This is the case especially for non-binary individuals, as they usually desire to subsidize the development of the sex characteristics related to their sex assigned at birth without implementing further body alterations. The data on the effect of puberty suppression for transgender people, albeit limited, showcase a clear improvement on mental health and quality of life in patients (AIR Miesen, 2020; Fisher et al., 2016; Grift et al., 2017). However, long-term studies in larger cohorts are needed in order to evaluate the results over time (Heijer et al., 2017; Leerdam et al., 2021).

1.4 GENDER-AFFRIMING HORMONE TREATMENT

Gender-affirming or cross-sex hormone treatment (GAHT) has been shown to have positive physical and psychological impact on individuals with GD. The treatment consists of gradually increasing doses of synthetic sex steroids in order to induce the development of the sex characteristics of the individual's identified gender. However, there is not a standard protocol for GAHT, as the regime is designed based on each individual's needs and health risk status (Unger, 2016). In general, sex steroids will affect growth and bone development, insulin sensitivity as well as lipid metabolism (Hembree, Cohen-Kettenis, Delemarre-van de Waal, et al., 2009).

AFABs are administered testosterone, more specifically testosterone esters, to suppress female secondary sex characteristics and induce virilization (Unger, 2016). Androgens (testosterone and 5α-dihydrotestosterone) cause a feedback loop reaction from the gonads both on the hypothalamic and pituitary level (Fig 2). At the hypothalamus, testosterone inhibits GnRH synthesis and secretion into the hypophyseal portal circulation. The effects of testosterone and especially 5α-DHT on pituitary function include the inhibition of LH production and secretion in a dose and time-dependent manner, as well as the increase of FSH synthesis and secretion basal levels (Fig 2) (Durán-Pastén & Fiordelisio, 2013). .

In AMABs, exogenous estrogen is used to alter fat distribution, and induce breast formation. The administration of the natural 17β estradiol (Fig 3) is preferred over its synthetic estrogen counterparts such as ethinyl estradiol, which used to be the standard treatment, to prevent any possible thrombogenesis (Claahsen - van der Grinten et al., 2020). Estradiol acts directly on the a-estrogen receptor of the anterior pituitary (Fig 2) increasing the expression of the pituitary GnRH receptor, causing a decrease in the GnRH concentration levels required for the threshold response of the FSH and LH secretion (Durán-Pastén & Fiordelisio, 2013).

It is advised that during GAHT, the administration of puberty suppressors is continued to maintain full suppression of pituitary gonadotropin levels and thereby, endogenous gonadal steroids. Otherwise, the exogenous sex steroids of GAHT could potentially cause reactivation of gonadotropin secretion leading to endogenous gonadal steroids release, rendering the GAHT less effective (Hembree, Cohen-Kettenis, Delemarre-van de Waal, et al., 2009). Another advantage of early GnRHa-caused pituitary inhibition is that lower doses of exogenous hormones can be administrated during the treatment. For AMABs, GnRHa are preferred over other anti-androgens such as cyproterone acetate or spironolactone (Fig 3), as there is no published evidence of the efficiency of exogenous synthetic sex steroids on gonadal axis suppression during puberty. As for AFABs, it is advised to receive GnRH for the time period necessary to reach the maintenance dosage of testosterone (Hembree et al., 2017).

Assigned-Female at Birth Patients	Dosage
Testosterone	
Oral: testosterone undecanoate	160–240 mg/d
Parenteral	
Testosterone enanthate or cypionate	100–200 mg IM every 2 weeks or 50% weekly
Testosterone undecanoate	1000 mg every 12 weeks
Transdermal	
Testosterone gel 1%	2.5–10 g/d
Testosterone patch	2.5–7.5 mg/d

Assigned Male at Birth Patients	Dosage
Estrogen	
Oral: estradiol	2.0–6.0 mg/d
Transdermal: estradiol patch	0.1–0.4 mg twice weekly
Parenteral: estradiol valerate or cypionate	5–20 mg IM every 2 weeks or 2–10 mg IM every week
Antiandrogens	
Spironolactone	100–200 mg/d
Cyproterone acetate	50–100 mg/d
GnRH agonist	3.75 mg SC monthly

Figure 3. Examples of gender-affirming hormone treatment and protocol for individuals with gender dysphoria. IM: intramuscularly, SC: subcutaneously Adapted from (Hembree, Cohen-Kettenis, Delemarre-van de Waal, et al., 2009).

1.5 STRUCTURAL CEREBRAL SEX DIMORPHISM

GD has long been theorized to be attributed to differential cerebral sexual differentiation with subcortical regions displaying volume differences based on sex (sex dimorphism) (Dörner, 1988; Swaab et al., 1995). The hypothesis of brain sex dimorphism was based on studies suggesting that cisgender (individuals whose gender identity aligns with their sex assigned at birth) women display larger hippocampal and caudate volumes, as well as smaller amygdala, putamen, and thalamus volumes in comparison to cis men (I Savic & Arver, 2013). The aforementioned brain structures were monitored due to their high androgen receptor expression as they could potentially display the effects of testosterone treatment (Lentini et al., 2013). It has been reported that testosterone levels show a negative correlation with parietal cortex thickness (Cth) and a positive one with fractional anisotropy (FA) values (FA reflecting white matter connections) (Inano et al., 2011; I Savic & Arver, 2013). In addition, when exploring the white matter (WM) and grey matter (GM) fraction, cis men displayed larger GM volumes (gray matter volume is a composite metric of Cth and surface area) in the cerebellum and lingual gyrus and smaller GM and WM volumes in the precentral gyrus than cis women (Ivanka Savic & Arver, 2011). However, there is no direct correlation between the volume of GM or WM and function, as their diverse composition from neurons, glial cells, myelin, dendrites, axons and synapses inhibits any determined causal link (Ivanka Savic & Arver, 2011).

1.6 SELF-BODY PERCEPTION NETWORKS AND CEREBRAL FUNCTIONAL DIMORPHISM

Research on GD has shifted towards the unravelling of neuronal networks that process the perception of self (“self-referential”) and those that mediate own-body perception (self-body perception networks). As

the main feature of GD is based on the discomfort with one's own body and estrangement feelings towards one's physical sex, it would be heavily speculated that the incongruence between one's sense of self and one's body relied on the self-body perception networks (Khorashad et al., 2021). Self-body perception is associated with the default mode network (DMN) and salience network (SN) (Fig 4). The DMN consists of the medial prefrontal cortex (mPFC), precuneus and bilateral parietal cortices, which are involved in self-other decision making. The SN consists of the fronto-insular and the pregenual anterior cingulate cortex (pACC) which both play a role in facilitating decision about self-representation (Fig 4) (Feusner et al., 2017). The pACC is of particular interest, as it is involved in self-conscious emotional processing, highlighting its importance in the sense of self neuronal circuits (Murray et al., 2015).

In addition, regions of the occipital cortex which is associated with visual attention and in this case for the visual processing of static bodies are implicated in self-body perception; the extrastriate and fusiform body areas (EBA and EFB accordingly) (Feusner et al., 2017). The EBA of the extrastriate lateral occipital body is linked to the frontal cortices through the fronto-occipital tract whose fibers go through the occipital and temporal lobes (David et al., 2007; Sarubbo et al., 2011). Research has been focusing on these circuits, when assessing the activation patterns in participants for Body Morph tests which require the visual cues to interpret and process the images of their bodies, distorted at various degrees, and the DMN or SN to establish the degree to which they identify the image projected with their body or sense of self.

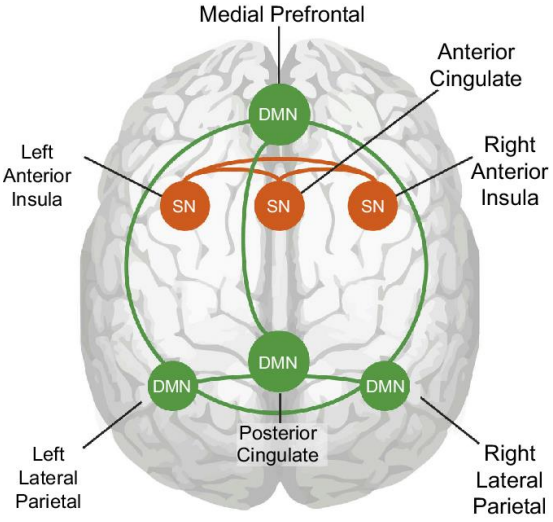

Figure 4. Node connections of the default mode network (DMN) and the salience network (SN). Green shows the connections between the nodes of the DMN and orange the connection between the nodes of the SN. Abbreviations: DMN, default mode network; SN, salience network (Van Ettinger-Veenstra et al., 2019)

Sex hormones have been reported to having differential effects on brain connectivity due to their receptor localization in the brain. Reportedly, there are androgen receptors expressed in both occipital and insular cortices, as well as certain subcortical volumes involved in emotional processing, motivation and reward, such as the amygdala and thalamus (Burke et al., 2018). Administration of estrogen to women and rats increases amygdala–PFC connectivity and higher estrogen levels are linked to increased functional connectivity, whereas it was shown that high endogenous testosterone levels in adolescents would exert the opposite effect (Khorashad et al., 2021). Higher amygdala–PFC connectivity could be related to higher emotional prevalence in the process of decision making or greater interpretation skills of emotional

valence or the commonly known emotional intelligence. Interestingly, studies in trans individuals (both AFAB and AMAB) showed greater cortical thickness (Cth) in the mPFC and bilaterally in the parieto-occipital cortex and weaker structural and functional connections in ventromedial PFC and pACC of the DMN compared with cisgender controls (Feusner et al., 2017; Manzouri & Savic, 2019). These findings support the hypothesis for the involvement of circuits incorporated in the DMN and SN and confirm the importance of own-body self-processing in GD.

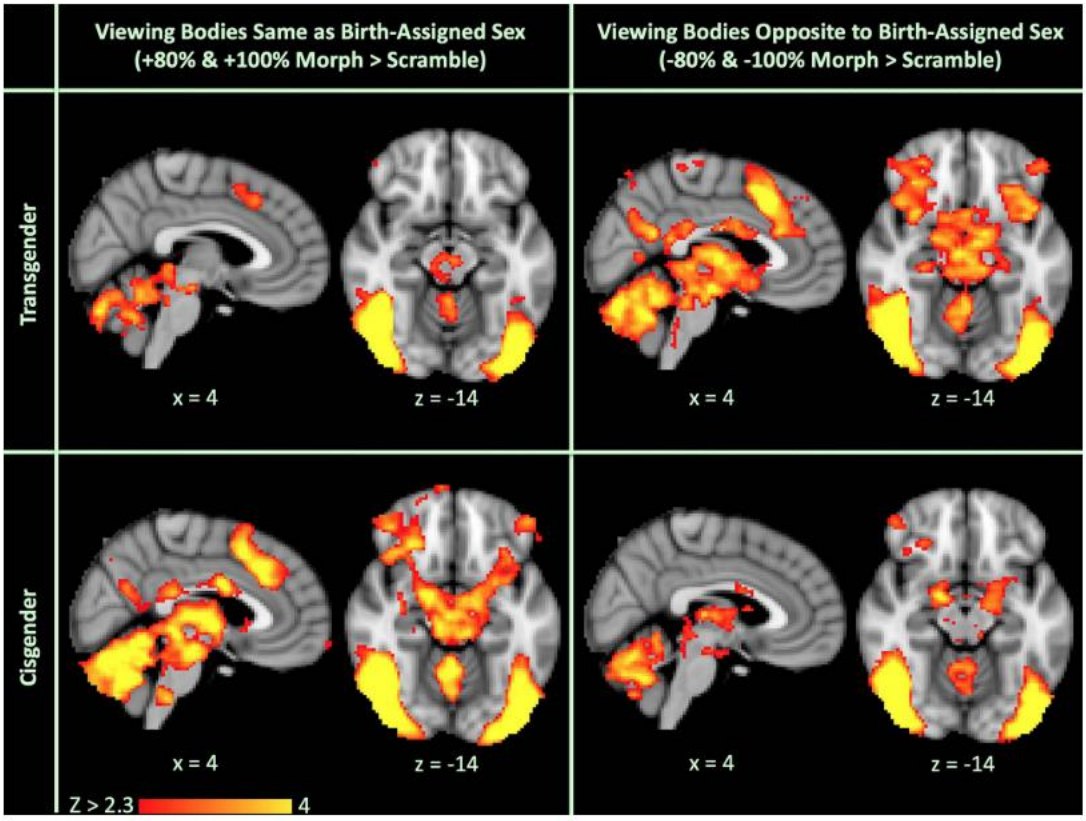

Figure 5. Comparison of fMRI activation maps in both transgender (upper) and cisgender (lower) groups when viewing other bodies same as birth-assigned sex (left panel) vs. other bodies opposite to birth-assigned sex (right pannel) compared to scrambled images in the long viewing condition after covarying out self- similarity ratings (Majid et al., 2020).

In order to elucidate possible functional cerebral sex differences, studies would measure with MRI scans the participants' response upon viewing distorted images of one's own body, either appearing with different degrees of thinness or fatness (body morphing) (Khorashad et al., 2021). Recently, a study compared the brain connectivity and activation between transgender and cisgender participants based on the body morph paradigm with own-body images displaying more masculine or feminine body characteristics (Majid et al., 2020). As expected, cisgender participants identified with images of themselves while transgender individuals identified with images morphed "opposite" to their birth-assigned sex. When examining their functional brain activation and taking the effect of self-similarity ratings into consideration, it was evident that in both groups the self-body neuronal processing systems aligned with their gender identity rather than their birth-assigned sex when examining the fMRI activation patterns (Fig 5) (Majid et al., 2020).

RESEARCH QUESTION

Gender dysphoria is gaining scientific interest in the most recent years, as individuals with GD are increasing in numbers mirroring a demand on the treatment alleviating the symptoms of GD. Clinically, patients with GD, transgender people, are treated with gender-affirming hormone treatment to enhance the biological sex characteristics of their identified gender. Until this day, the underlying neurobiological mechanisms behind GD remain unknown and for many years, research has been associating GD with altered cerebral sex dimorphism. However, the principle feature of GD, which is the strong perception of incongruence between one's sense of self and one's body and a feeling of discomfort with one's body sex phenotype, was not being addressed. Therefore, a shift in research of GD is observed among research teams investigating the functional aspect of brain dimorphism via focusing on neuronal circuits behind self-body perception.

This essay aims to investigate the effect of cross-sex hormone treatment on the brain from a structural and functional perspective, as reported from studies with transgender people diagnosed with gender dysphoria. In addition, specific interest will be shown in neuronal circuit activation and connectivity mediating the self ("self-referential") and own-body perception processes in an effort to gain further insight into the mechanisms underlying GD.

RESULTS

Structural connectivity

In the first part of the results, the effects of sex hormones on brain structure, more specifically, Cth and FA values were assessed. It has been reported that transgender individuals show greater cortical thickness than cisgender participants in studies before the start of their treatment (Manzouri et al., 2017; Manzouri and Savic 2019). Upon 6 months of testosterone administration, multiple brain regions displayed higher Cth, among them the left lingual, the inferior parietal, postcentral regions, as well as increased right thalamus volume in trans AFABs (Zubiaurre-Elorza et al., 2014). Another study following up on trans people after 4 months of GAHT showed that testosterone in trans AFABs led to an increase in total brain volume and the maintenance of the hypothalamus volume, whereas in cisgender AFABs, the hypothalamic volume was significantly reduced over time (Hulshoff Pol et al., 2006). In trans AMABs upon 4 months of estradiol treatment, the hypothalamic volume was decreased in contrast to the increase of ventricle volumes (Hulshoff Pol et al., 2006). In addition, trans AMABs display smaller insular grey matter volume pre and post estrogen treatment in relation to cisgender female controls (Spizzirri et al., 2018).

A more recent study aiming to measure the whole brain effects of testosterone 3 month administration in a small group of trans AFABs showed that the treatment led to an increase in Cth in the insular cortex and no significant changes in subcortical structures of the thalamus or amygdala (Burke et al., 2018). Trans men were reported with thicker mPFC than controls pre- and post-treatment (Burke et al., 2018). When an additional study was conducted from the same research group with a larger participant pool for both AFABs and AMABs, it showed that the regional Cth pattern was largely in accordance with the sex assigned at birth and confirmed the previous findings of the positive effect of testosterone on the medial temporal and insular cortices in trans men (Kilpatrick et al., 2019). The Cth increase was localized mainly in the insular and superior temporal cortex which are brain regions rich in testosterone receptor, as well as the frontal cortex In trans women, anti-androgen and estrogen treatment led to widespread cortical thinning (Kilpatrick et al., 2019). They also reported alterations in total grey and white matter volumes; increase

with testosterone; decrease with anti-androgen and estrogen treatment, aligned with findings from previous aforementioned studies. However, after correction for treatment-related changes in total grey and white matter volumes, both transgender groups showed a relative thinning of the right mPFC, left lateral temporo-occipital and parietal cortices, in contrast to their increased pre-treatment values (Fig 6) (Kilpatrick et al., 2019). Therefore, the initial divergence from controls at the frontal, occipito-parietal Cth disappeared upon the 6-month period of GAHT (Kilpatrick et al., 2019). In the same study, when rating the own-body congruence among participants, it was evident that the ratings increased with the treatment and, interestingly, the increase partially correlated with a left parietal cortical thinning, supporting the data so far with the positive results of GAHT (Kilpatrick et al., 2019).

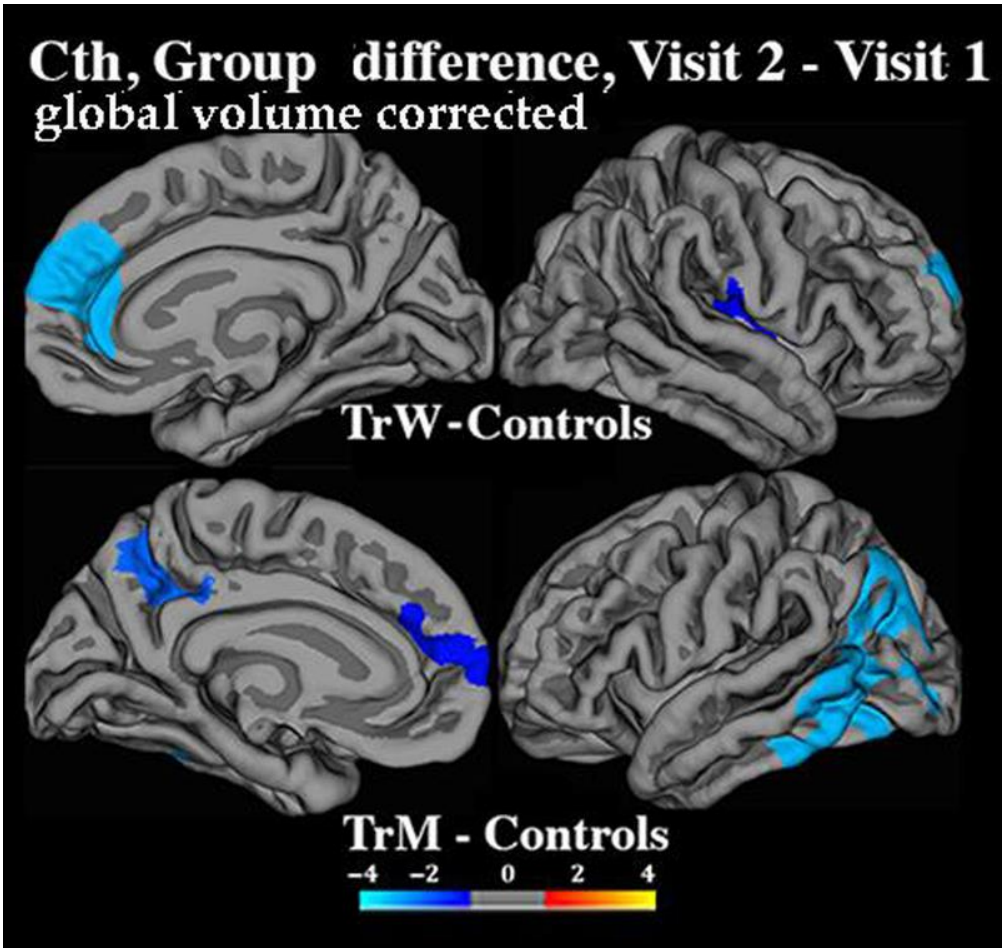

Figure 6. Comparison of cortical thickness pre- and post sex hormone treatment between trans participants and cisgender controls with correction for total WMV and GMV changes. The contrasts were calculated at $p < 0.05$. the Projection of cerebral hemispheres (MR images of the FreeSurfer atlas) is standardized. Scale is logarithmic and with cool colours indicating negative contrast, warm colours indicating positive contrast. TrW = transgender women; TrM = transgender men Visit 1; pre-treatment and Visit 2' post-treatment (Kilpatrick et al., 2019).

As for fractional anisotropy values, measured by Diffusion Tensor Imaging (DTI) upon 7 months on testosterone, trans men (trans AFABs) showed an increase in FA values in the right superior longitudinal, corticospinal and the fronto-occipital tract in comparison to pre-testosterone results (Rametti et al., 2012). This finding was confirmed in the same tracts when compared to cis AFABs controls and it was correspondent to cis male controls levels (Rametti et al., 2012). In accordance to this, a more recent

study indicated that 3-month testosterone administration led to right hemispheric increased FA in the tract connecting the mPFC and the occipital cortex (Burke et al., 2018).

Functional connectivity

Studies measuring functional connectivity scanned participants twice; the first session was established as pre-treatment and the second one followed after a determined period of sex hormone treatment. As mentioned above, the research focus was on the own-self processing networks, the DMN and SN. Connectivity patterns within the DMN showed a sub-significant increase in mPFC post-treatment in both trans men and women, whereas in controls a significant decrease in connectivity was reported in the same area (Khorashad et al., 2021). Additionally, in trans men, an increase in precuneus connectivity was observed (Khorashad et al., 2021). As for the SN, MRI scans revealed a significant connectivity increase in the mPFC, which was partly overlapping with a cluster in DMN, and the insular cortex in both transgender groups, but not in controls (Khorashad et al., 2021). Of note, trans women also displayed a significant increase in connectivity in the mPFC/ACC in comparison to controls (Khorashad et al., 2021). In line with these findings, a study incorporating only trans men pre- and post-testosterone treatment pointed towards increased functional connectivity, post-treatment, between parietal and frontal regions (mPFC and temporo-parietal junction-TPJ) involved in own body identification in the context of self, in comparison to controls (Fig 7) (Burke et al., 2018). As mentioned in the introduction, these regions displayed weaker connections pre-treatment in trans participants. Taking these data into consideration, GAHT seems to "restore" the communication among regions involved in own-body processing (Burke et al., 2018).

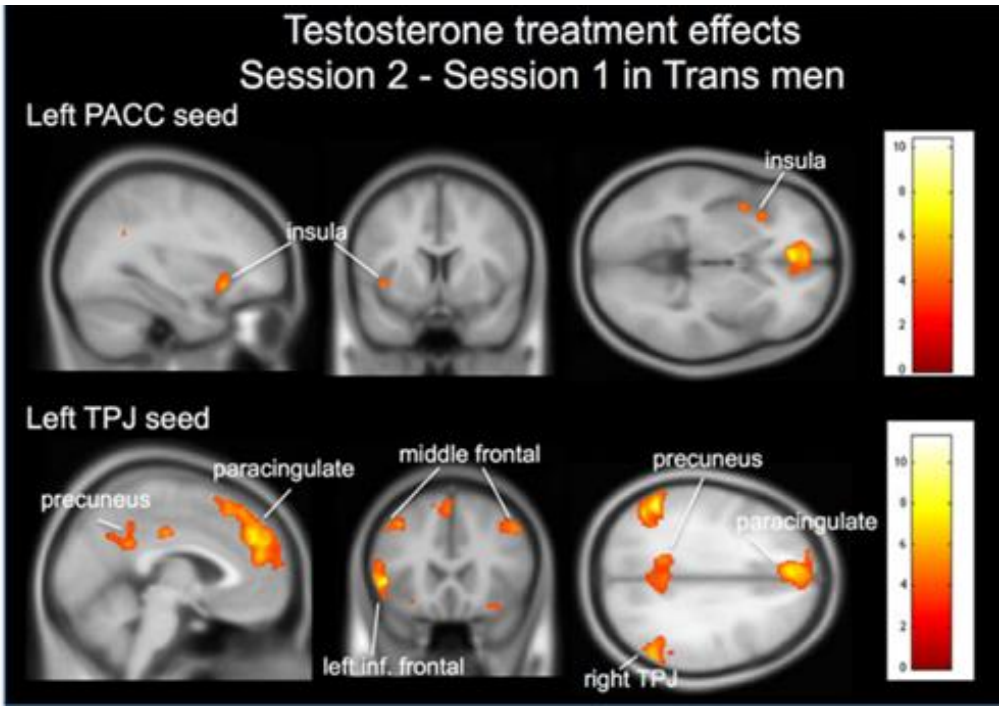

Figure 7. Testosterone treatment effects on functional connectivity between session 1 and 2 in trans men as depicted via MRI scan. Paired t-test results show increases in functional connectivity with treatment for the left pACC and TPJ. These areas linked to networks processing own body perception in the context of self, which displayed initially weaker functional connections, show significantly increased connectivity with testosterone treatment. Color bars indicate t-statistic. Modified based on (Burke et al., 2018).

Own-Body perception task

The effect of sex hormone treatment on own-body perception for people with GD was assessed via the Body Morph test with results in agreement with the studies presented in this essay (see 1.6) (Fig 8). Prior to treatment, trans participants (AFABs and AMABs) rated significantly lower their unmorphed images as "self", since they would not identify themselves with that image (Fig 8) than cis controls (Khorashad et al., 2021). During their second scan, upon the completion of their treatment, a significant increase in the 0% ratings was noted in both trans men with testosterone and trans women with estrogen treatment in comparison to their pre-treatment ratings (Khorashad et al., 2021). Additionally, the initial difference between trans and cisgender participants was eliminated at the second scan as well (Khorashad et al., 2021)

Another study combining MRI scans while the participants who had completed GAHT were engaged in the Body Morph test confirmed Khorasad et al findings and shed more light into the activation of specific brain regions during the scans. When viewing images morphed "toward" the participants' birth-assigned sex, there was no significant difference between groups. In contrast, during opposite to birth-assigned sex images viewing, there was a significant group effect, with transgender individuals, both AFABs and AMABs, showing significant greater "self" association (Majid et al., 2020). The ratings were accompanied with the activation in the in dorsomedial prefrontal cortex (dmPFC), including the pACC and paracingulate gyrus (Majid et al., 2020). The same activation pattern was observed in transgender participants pre-treatment. Interestingly, upon viewing androgynous morphs (−40% and −60% morphs), transgender participants displayed significantly higher connectivity than cisgender controls between the pACC and the areas of the visual cortex partially overlapping with the EBA (Majid et al., 2020).

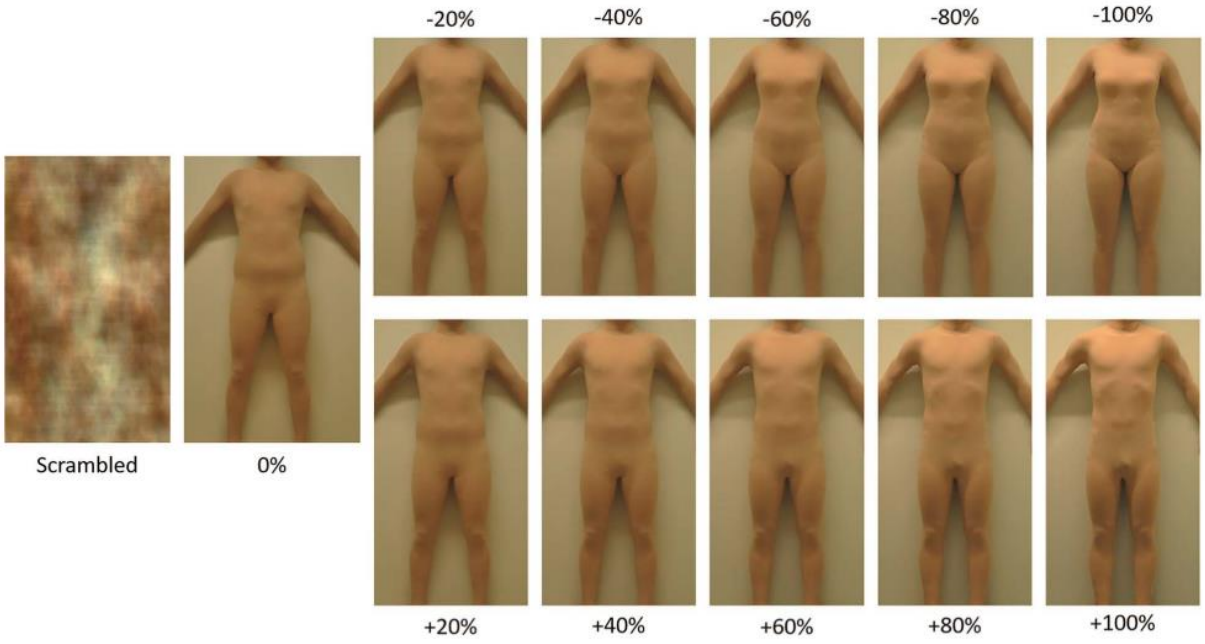

Figure 8. Example of a Body morph test. In this figure, the participant's own body (male) is represented at 0% morph degree and another of the opposite sex assigned at birth at 100%. In between morphing degrees either positive for morphing towards the same and negative for the opposite sex in relation to the participant's birth-assigned sex (Majid et al., 2020).

DISCUSSION

This essay aimed to present studies focusing on the effect of gender-affirming hormone treatment on the brain of people with gender dysphoria both from a structural and functional perspective. Literature cites increased Cth in the mPFC, pACC and precuneus in TrW, as well as in TrM, which are regions implicated in own-body perception networks (Feusner et al., 2017; Manzouri & Savic, 2019). Studies on the effect of cross sex hormone treatment on cortical thickness confirmed previous findings (Zubiaurre-Elorza et al., 2014; Burke et al., 2018); Estrogen treatment led to widespread decreases in Cth in trans AMABs, which could be associated to exerted effects of estrogen on the PFC, cerebellum, hippocampus and the primary sensory-motor cortex (Hao et al., 2007; Kilpatrick et al., 2019). In contrast, testosterone resulted in more localized increases in Cth in regions with high testosterone-receptors distribution such as the insular and superior temporal cortices and to a lesser extent, in the frontal cortex (Kilpatrick et al., 2019). As for cortical volumes alterations, studies reported similar results with testosterone increasing both GMV and WMV, whereas estrogen and anti-androgen treatment had the opposite effect, at a greater degree even in the GMV (Hulshoff Pol et al., 2006; Perrin et al., 2008; Spizzirri et al., 2018; Kilpatrick et al., 2019). However, upon correlation of Cth with the cortical volumes, it appeared that regions such as the right mPFC, left lateral temporo-occipital and parietal cortices, which are associated with self-perception processing, displayed cortical thinning reaching the levels of cisgender participants (Kilpatrick et al., 2019).

In accordance to the testosterone-induced cortical changes, trans men upon testosterone treatment displayed significant increases in FA values, which reflect white matter connectivity, in the fronto-occipital tract (Rametti et al., 2012; Burke 2018). This circuit connects the mPFC with the EBA of the occipital cortex and conveys visual information, which could be related to own-body perception, thus suggesting a stronger self-referential concept for trans individuals (David et al. 2007; Burke et al., 2018). Unfortunately, no published study was found investigating the effects of estrogen treatment on structural connectivity in trans women.

In line with the structural connectivity findings, functional connectivity seems to be increased by sex hormone treatment among regions implicated in body perception and self-referential processing. Mainly, research focused on the self-referential network, default mode network (DMN) associated with areas such as the mPFC, precuneus, the left angular gyrus and superior parietal cortex as well as the pACC of the salience network (SN) involved in self-perception and identification. Gender affirming hormone treatment was shown to increase connectivity among these regions mediating own-body perception both in trans men and women as shown in fMRI scans (Khorashad et al., 2021). Additionally, self-reported identification with their body phenotype would increase upon treatment in both trans men and women (Khorashad et al., 2021). In support of this, another study in trans men showed that testosterone treatment restored the functional connectivity between parietal and frontal regions (mPFC and temporo-parietal junction-TPJ) involved in own-body identification in the context of self, which was significantly weaker pre-treatment in comparison to controls (Fig 7) (Burke et al., 2018).

Taking into account the brain region pattern activation during the body morph tests, transgender participants would activate similar self-referential networks involving the dmPFC, when viewing images aligning with their gender identity and not the sex assigned at birth (Majid et al., 2020). Interestingly, a study with women suffering from anorexia nervosa receiving low-dose testosterone treatment over a 3-week period reported treatment-induced metabolism of the pACC, right caudate nucleus and a cluster in the right parietal lobe, close to the TPJ (Miller et al. 2004). There is an aspect of correlation concerning

the effect of testosterone in the brain between these two conditions; gender dysphoria and anorexia nervosa, both characterized by feelings of body-phenotype distress and body-related avoidance. These data could support the hypothesis that GD is associated with specific anatomical features in own-body/self-processing circuits that reverse to the pattern of cisgender controls after cross-sex hormone treatment.

So far, the aforementioned studies support that gender-affirming hormone treatment is able to alter the connectivity among brain regions and circuits mediating self-other perception. It is evident that the results on their physical appearance led by the treatment contribute to the alleviation of body dysphoria in transgender individuals, as they identify at a greater degree with their body image. It is suggested that this change is mirrored on the increased congruence with their own-body perception and thus functional connectivity within the circuits of the mPFC and the parietal cortex. It remains to be elucidated whether the hormonal treatment is primarily causal to the increased functional brain connectivity and the interplay behind the self-referential networks alteration in gender dysphoria.

FUTURE PERSPECTIVES

Research on gender dysphoria is in its early steps, therefore there is great room for amelioration. Thus far, there was not necessarily gender equality as far as the participants are concerned in most studies discussed in this essay. Therefore, it would be scientifically more accurate to include equal numbers of transgender AFABs and AMABs, even though finding willing participants can be difficult. On a similar aspect, it is pivotal to conduct more research on the effect of sex hormone treatment on trans AMABs, as a higher number of studies at the moment is focused on testosterone effects on AFABs. In addition, it would be interesting to investigate the activation patterns of self-perception in trans non-binary individuals, as it is a group that is often disregarded due to binary classification purposes, even though it consists of a large part of the transgender population. It is important for future studies to focus on own-body perception networks and incorporate fMRI scans with the Body Morph test while exploring both conditions of pre- and post-treatment in order to acquire a more clear idea about the neural circuits behind gender dysphoria. Last but not least, it is worth noting that the effects of cross-sex hormone treatments on the brain have been investigated within a small timeframe (3-7 months) upon treatment completion. Thus, it would be beneficial to scan participants in longer period of time as well, in order to be able to elaborate on the longitudinal effects of gender-affirming hormone treatment on people with gender dysphoria.

BIBLIOGRAPHY

AIR Miesen, T. S. A. V. H. B. A. P. (2020). Psychological functioning in transgender adolescents before and after gender-affirmative care compared with cisgender general population peers. *J Adolesc Health*, *66*(6), 699–704. https://doi.org/10.1016/j.jadohealth.2019.12.018

Burke, S. M., Majid, D. S. A., Manzouri, A. H., Moody, T., Feusner, J. D., & Savic, I. (2019). Sex differences in own and other body perception. *Human Brain Mapping*, *40*(2), 474–488. https://doi.org/10.1002/hbm.24388

Burke, S. M., Manzouri, A. H., Dhejne, C., Bergström, K., Arver, S., Feusner, J. D., & Savic-Berglund, I. (2018). Testosterone Effects on the Brain in Transgender Men. *Cerebral Cortex*, *28*(5), 1582–1596. https://doi.org/10.1093/cercor/bhx054

Claahsen - van der Grinten, H., Verhaak, C., Steensma, T., Middelberg, T., Roeffen, J., & Klink, D. (2020). Gender incongruence and gender dysphoria in childhood and adolescence—current insights in diagnostics, management, and follow-up. *European Journal of Pediatrics 2020 180:5*, *180*(5), 1349–1357. https://doi.org/10.1007/S00431-020-03906-Y

Claahsen - van der Grinten, H., Verhaak, C., Steensma, T., Middelberg, T., Roeffen, J., & Klink, D. (2021). Gender incongruence and gender dysphoria in childhood and adolescence—current insights in diagnostics, management, and follow-up. *European Journal of Pediatrics*, *180*(5), 1349–1357. https://doi.org/10.1007/s00431-020-03906-y

Cohen-Kettenis, P. T., & Pfäfflin, F. (n.d.). *The DSM Diagnostic Criteria for Gender Identity Disorder in Adolescents and Adults*. https://doi.org/10.1007/s10508-009-9562-y

David, N., Cohen, M. X., Newen, A., Bewernick, B. H., Shah, N. J., Fink, G. R., & Vogeley, K. (2007). The extrastriate cortex distinguishes between the consequences of one's own and others' behavior. *NeuroImage*, *36*(3), 1004–1014. https://doi.org/10.1016/J.NEUROIMAGE.2007.03.030

Dörner, G. (1988). Neuroendocrine response to estrogen and brain differentiation in heterosexuals, homosexuals, and transsexuals. *Archives of Sexual Behavior*, *17*(1), 57–75. https://doi.org/10.1007/BF01542052

Durán-Pastén, M. L., & Fiordelisio, T. (2013). GnRH-induced Ca2+ signaling patterns and gonadotropin secretion in pituitary gonadotrophs. Functional adaptations to both ordinary and extraordinary physiological demands. *Frontiers in Endocrinology*, *4*(SEP). https://doi.org/10.3389/FENDO.2013.00127

Feusner, J. D., Lidström, A., Moody, T. D., Dhejne, C., Bookheimer, S. Y., & Savic, I. (2017). Intrinsic network connectivity and own body perception in gender dysphoria. *Brain Imaging and Behavior*, *11*(4), 964. https://doi.org/10.1007/S11682-016-9578-6

Fisher, A. D., Castellini, G., Ristori, J., Casale, H., Cassioli, E., Sensi, C., Fanni, E., Amato, A. M. L., Bettini, E., Mosconi, M., Dèttore, D., Ricca, V., & Maggi, M. (2016). Cross-Sex Hormone Treatment and Psychobiological Changes in Transsexual Persons: Two-Year Follow-Up Data. *The Journal of Clinical Endocrinology & Metabolism*, *101*(11), 4260–4269. https://doi.org/10.1210/JC.2016-1276

Grift, T. C. van de, Elaut, E., Cerwenka, S. C., Cohen-Kettenis, P. T., Cuypere, G. De, Richter-Appelt, H., & Kreukels, B. P. C. (2017). Effects of Medical Interventions on Gender Dysphoria and Body Image: A Follow-Up Study. *Psychosomatic Medicine*, *79*(7), 815. https://doi.org/10.1097/PSY.0000000000000465

Heijer, M. den, Bakker, A., & Gooren, L. (2017). Long term hormonal treatment for transgender people. *BMJ*, *359*. https://doi.org/10.1136/BMJ.J5027

Hembree, W. C., Cohen-Kettenis, P., Delemarre-van de Waal, H. A., Gooren, L. J., Meyer, W. J., Spack, N. P., Tangpricha, V., & Montori, V. M. (2009). Endocrine Treatment of Transsexual Persons:An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, *94*(9), 3132–3154. https://doi.org/10.1210/JC.2009-0345

Hembree, W. C., Cohen-Kettenis, P., Delemarre-Van De Waal, H. A., Gooren, L. J., Meyer, W. J., Spack, N. P., Tangpricha, V., & Montori, V. M. (2009). Endocrine treatment of transsexual persons: An endocrine society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*, *94*(9), 3132–3154. https://doi.org/10.1210/JC.2009-0345

Hembree, W. C., Cohen-Kettenis, P. T., Gooren, L., Hannema, S. E., Meyer, W. J., Murad, M. H., Rosenthal, S. M., Safer, J. D., Tangpricha, V., & T'Sjoen, G. G. (2017). Endocrine treatment of gender-dysphoric/ gender-incongruent persons: An endocrine society∗clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism*, *102*(11), 3869–3903. https://doi.org/10.1210/JC.2017-01658

Inano, S., Takao, H., Hayashi, N., Abe, O., & Ohtomo, K. (2011). Effects of age and gender on white matter integrity. *AJNR. American Journal of Neuroradiology*, *32*(11), 2103–2109. https://doi.org/10.3174/AJNR.A2785

J Ristori, T. S. (2016). Gender dysphoria in childhood. *Int Rev Psychiatr*, *28*(1), 13–20. https://doi.org/10.3109/09540261.2015.1115754

Jones, B. A., Pierre Bouman, W., Haycraft, E., & Arcelus, J. (2019). Gender congruence and body satisfaction in nonbinary transgender people: A case control study. *International Journal of Transgenderism*, *20*(2–3), 263–274. https://doi.org/10.1080/15532739.2018.1538840

Khorashad, B. S., Manzouri, A., Feusner, J. D., & Savic, I. (2021). Cross-sex hormone treatment and own-body perception: behavioral and brain connectivity profiles. *Scientific Reports*, *11*(1), 1–11. https://doi.org/10.1038/s41598-020-80687-2

Leerdam, T. R. van, Zajac, J. D., & Cheung, A. S. (2021). The Effect of Gender-Affirming Hormones on Gender Dysphoria, Quality of Life, and Psychological Functioning in Transgender Individuals: A Systematic Review. *Https://Home.Liebertpub.Com/Trgh*. https://doi.org/10.1089/TRGH.2020.0094

Leinung, M. C., Urizar, M. F., Patel, N., & Sood, S. C. (2013). Endocrine Treatment of Transsexual Persons: Extensive Personal Experience. *Endocrine Practice* , *19*(4), 644–650. https://doi.org/10.4158/EP12170.OR

Lentini, E., Kasahara, M., Arver, S., & Savic, I. (2013). Sex Differences in the Human Brain and the Impact of Sex Chromosomes and Sex Hormones. *Cerebral Cortex*, *23*(10), 2322–2336. https://doi.org/10.1093/CERCOR/BHS222

Majid, D. S. A., Burke, S. M., Manzouri, A., Moody, T. D., Dhejne, C., Feusner, J. D., & Savic, I. (2020). Neural Systems for Own-body Processing Align with Gender Identity Rather Than Birth-assigned Sex. *Cerebral Cortex*, *30*(5), 2897–2909. https://doi.org/10.1093/cercor/bhz282

Manzouri, A., & Savic, I. (2019). Possible Neurobiological Underpinnings of Homosexuality and Gender Dysphoria. *Cerebral Cortex (New York, NY)*, *29*(5), 2084. https://doi.org/10.1093/CERCOR/BHY090

NM Graaf, P. C. T. S. K. Z. (2018). Evidence for a change in the sex ratio of children referred for gender

dysphoria: data from the gender identity development service in London (2000-2017). *J Sex Med*, *15*(10), 1381–1383. https://doi.org/10.1016/j.jsxm.2018.08.002

Roth, C. L., Brendel, L., Rückert, C., & Hartmann, K. (2005). Antagonistic and Agonistic GnRH Analogue Treatment of Precocious Puberty: Tracking Gonadotropin Concentrations in Urine. *Hormone Research in Paediatrics*, *63*(5), 257–262. https://doi.org/10.1159/000086685

Sarubbo, S., De Benedictis, A., Maldonado, I. L., Basso, G., & Duffau, H. (2011). Frontal terminations for the inferior fronto-occipital fascicle: anatomical dissection, DTI study and functional considerations on a multi-component bundle. *Brain Structure and Function 2011 218:1*, *218*(1), 21–37. https://doi.org/10.1007/S00429-011-0372-3

Savic, I, & Arver, S. (2013). *Sex Differences in Cortical Thickness and Their Possible Genetic and Sex Hormonal Underpinnings*. https://doi.org/10.1093/cercor/bht180

Savic, Ivanka, & Arver, S. (2011). Sex Dimorphism of the Brain in Male-to-Female Transsexuals. *Cerebral Cortex*, *21*(11), 2525–2533. https://doi.org/10.1093/CERCOR/BHR032

Standards of Care for the Health of Transsexual, Transgender, and Gender Nonconforming People The World Professional Association for Transgender Health. (n.d.). Retrieved November 2, 2021, from www.wpath.org

Swaab, D. F., Gooren, L. J. G., & Hofman, M. A. (1995). Brain Research, Gender and Sexual Orientation. *Journal of Homosexuality*, *28*(3–4), 283–301. https://doi.org/10.1300/J082V28N03_07

Unger, C. A. (2016). Hormone therapy for transgender patients. *Translational Andrology and Urology*, *5*(6), 877. https://doi.org/10.21037/TAU.2016.09.04

Van Ettinger-Veenstra, H., Lundberg, P., Alföldi, P., Södermark, M., Graven-Nielsen, T., Sjörs, A., Engström, M., & Gerdle, B. (2019). Chronic widespread pain patients show disrupted cortical connectivity in default mode and salience networks, modulated by pain sensitivity. *Journal of Pain Research*, *12*, 1743–1755. https://doi.org/10.2147/JPR.S189443

WC Hembree, P. C.-K. L. G. S. H. W. M. M. M. S. R. J. S. V. T. G. T. (2017). Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*, *102*(11), 3869–3903. https://doi.org/10.1210/jc.2017-01658

Zubiaurre-Elorza L, Junque C, Gómez-Gil E, Guillamon A. 2014. Effects of cross-sex hormone treatment on cortical thickness in transsexual individuals. J Sex Med. 11: 1248–1261.