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Variations in Sex Differentiation: The Neurobiology of Gender Dysphoria

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Abstract

The aim of this review paper was to investigate variations in sex differentiation, and also, examine what neurobiological underpinnings there are to gender identity and gender dysphoria. In addition, the most extreme form of gender dysphoria, transsexuality, will be described from a neurobiological perspective but also discussed in terms of the classification from DSM-5. One theory considered on how gender identity originates is the fact that the sexual differentiation of the brain and the differentiation of sexual organs develop during different time periods. Alterations were displayed in a demonstration of male-to-female (MTF) and female-to-male (FTM) transsexuals that showed reversed results in cell number in a part of the hypothalamus, acronymized INAH-3 and reversal volume results in another region, acronymized BSTc. Likewise, differences in grey matter in the right putamen depended upon their natal gender. It can be concluded that there is biological evidence for sex differentiation and indications that lead science into considering biological components for gender dysphoria. This conclusion suggests for future research questions focused more on the possible genetic factors of gender identity, also, consider larger sample sizes and more replications. There is still incomplete knowledge of what exactly constitutes an individual's gender identity.

Keywords: Gender identity, gender dysphoria, sex, differentiation, gender, transsexuality, neurodevelopment, INAH-3, BSTc

Table of Contents

1. Introduction

Long before Christian colonization, fluid gender identities of the Native American *Two-Spirits* were seen as a gift from the gods, which carried unique responsibilities that were vital for the collective well-being of Native American tribes (Hunt, 2016). In Samoa, *fa'afafine* terminology equals feminine males, who are treated with high social tolerance compared to Western societies' standards (Vasey & Bartlett, 2007). As found, altered expressions of gender and sexuality have been displayed throughout history. Moreover, the Danish artist Lili Elbe, born Einar Wegener was among the first-ever documented recipients of sex-reassignment surgery (SRS) in the 1920's by Magnus Hirschfeld (Hoyer, 2004). Likewise, the *Self-Portrait with Cropped Hair* by Frida Kahlo painted in 1940, portrays cross-dressing in modern arts. The famous black and white photographs profiling Frida Kahlo, with a quirky smile in the act of cross-dressing, have become historic in representation for the *transgender* (embracing individuals who are not comfortable with the gender identity assigned at birth) community.

Gender is such a familiar part of human daily life that assumptions and presuppositions are unconsciously produced in order for us to try to categorize and explain our surroundings (Lorber, 1994). Thus, how we handle gender is in some way constructed out of human interaction. Or, as Lorber (1994, p.54) puts it: "Talking about gender for most people is the equivalent of fish talking about water". *Gender identity* is the perception of what gender we belong to, which has been seen to extend beyond our biological sex (Luders et al., 2009). The sense of our self-experienced maleness or femaleness plays an important role in how we form our relationships with others but also how we form our relationship with ourselves (Ngun, Ghahramani, Sánchez, Bocklandt, & Vilain, 2011). Historically, insufficient knowledge and too

much speculation have led to great misconceptions concerning the lives of transgender individuals (Cohen-Kettenis & Gooren, 1999). Furthermore, in Western societies, a longing for SRS has frequently been considered a wish from a disturbed mind.

Gender dysphoria is a clinical term, which involves a disagreement between an individual's physical or assigned gender in relation to the gender which he/she/they identify (see appendix). Gender dysphoria ought to be seen as a spectrum disorder categorized by dissatisfaction with one's social gender, biological sex (or both), where the severity of the experienced distress varies (Blaney & Millon, 2008). Mild forms of gender dysphoria might sometimes involve inconsistent identification as male or female or sometimes both at the same time. *Transsexuality* is recognized as the most extreme form of gender dysphoria, where the longing for SRS and or hormonal treatments repetitively occurs (Bao & Swaab, 2011; Blaney & Millon, 2008; Cohen-Kettenis & Gooren, 1999; Garcia-Falgueras & Swaab, 2008; Korte et al., 2008; Kruijver et al., 2000; Luders et al., 2009; Rametti et al., 2011; Swaab, 2004). Being transsexual is independent of *sexual orientation* (American Psychiatric Association, 2013; Cohen-Kettenis & Gooren, 1999; Smith, Junger, Derntl, & Habel, 2015; Swaab, 2007). This means that transgender individuals may identify themselves as homosexual, bisexual, heterosexual etc., or may consider conventional sexual orientation labels inadequate in order to explain their sexuality. The term *transsexual* entered the literature in 1923, through the work of Magnus Hirschfeld (Cohen-Kettenis & Gooren, 1999; Hoyer, 2004). In the beginning, the term transsexualism was associated with transvestism and effeminate homosexuality. Its modern sense, the longing to live as the opposite sex, was established from the 1940s and onward.

In Sweden, the annual incidence of gender dysphoria has been expected to be

0.17 per 100,000 citizens (Landén, Wålinder, & Lundström, 1996; Swaab, Chung, Kruijver, Hofman, & Hestiantoro, 2003). Furthermore, the prevalence of gender dysphoria data in Sweden conducted in the 1990s was compared to reports on transsexualism in the 1960s and 1970s (also conducted in Sweden) and on average, the male: female in prevalence studies is estimated to be 1.4:1 (Landén et al., 1996). Compared to other countries, the sex ratio (i.e. ratio of genetic males to females in a population) varies from 1.4:1 to 3:1 (Landén et al., 1996; Swaab et al., 2003). The estimation of prevalence was conducted by counting patients seeking SRS. The data of this estimation lacks unrecorded numbers of individuals who do not seek out a diagnosis (American Psychiatric Association, 2013; Cohen-Kettenis & Gooren, 1999; Landén et al., 1996). Therefore, these numbers are unreliable and in need of revision. Further, due to differentiations in societal tolerance in dissimilar countries, exact prevalence rates are absent (Smith et al., 2015). According to the American Psychiatric Association (APA) (2013), the prevalence of gender dysphoria in natal adult females ranges from 0.002% to 0.003% and natal adult males from 0.005% to 0.014%. Sex ratio in young natal boys to girls ranges from 2:1 to 4.5:1. In adolescents, the sex ratio is more balanced between the sexes. Korte et al. (2008) propose prevalence of 1 % of the total population in children and adolescences.

Transsexualism first appeared as a diagnosis in *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-III around the 1980s proposed by APA (Cohen-Kettenis & Gooren, 1999). The term was later abandoned for the umbrella-term *Gender Identity Disorder,* which in turn replaced the term *Transsexualism* (Cohen-Kettenis & Gooren, 1999; Lev, 2013). A paradigm shift was made on the 1st of December 2012 when the Board of Trustees approved the final draft of the DSM-5 for APA (Lev, 2013). *Gender Identity Disorder* was replaced with the term *Gender*

Dysphoria, which also shifted the focus from gender nonconformity to more of a diagnosis on distress and dysphoria. The diagnostic features on gender dysphoria describe how the incongruence also needs to cause anguish to the patient (American Psychiatric Association, 2013). The distress does not have to be directed to simply be of the other gender, but may also include an alternative gender. In order to diagnose, there has to be some form of evidence of the incongruence being demanding (see appendix). Also, in DSM-5, the term *gender* is used since the term *sex* might have conflicting biological indicators of sex (such as being born with two types of sex). Additionally, the term *gender* is used to designate the lived role as male or female, in terms of social constructs (American Psychiatric Association, 2013).

Furthermore, gender dysphoria has separate criteria for adults and children. Gender dysphoria in children has criteria separated in categories A and B. Criterion A1 must be fulfilled for a diagnosis (see appendix). Furthermore, in adolescents and adults, the same premises of A and B are applied, however for a diagnosis to be determined it needs to be manifested by at lease two of the six criteria during section A and B (see appendix). Furthermore, DSM-5 claims *comorbidity* (where more than one diagnosis or illness is present in a single individual) in individuals diagnosed with transsexuality normal. Identified in prepubertal children, a lot of emotional or behavioral difficulties have been recognized. Increasing age is correlated with having more acute issues, caused by the limiting, societal gender expectations and non-acceptance by the surrounding milieu. The associated features supporting diagnosis describe how adolescents and adults who experience gender dysphoria before SRS or hormonal treatment belong to a group of expanded risk for suicidal thoughts and or suicidal attempts (American Psychiatric Association, 2013).

At present time, more conscious realization and scientific findings have been

brought to the field of gender identity and transsexuality (Cohen-Kettenis & Gooren, 1999; Zucker & Lawrence, 2009). However, incomplete knowledge of what are the exact mechanisms of gender identity remains an unsolved puzzle to this day. Neuroscientific studies have displayed variations in differentiation between genders. The results show clearly how different parts of the brain are involved in forming an individual's gender identity. This has led to a notable theory on why gender identity develops, which originates in the fact that the sexual differentiation of the brain and the differentiation of sexual organs develop during different time periods (Bao & Swaab, 2011; Berglund, Lindström, Dhejne-Helmy, & Savic, 2008; Chung, De Vries, & Swaab, 2002; Cohen-Kettenis & Gooren, 1999; Garcia-Falgueras & Swaab, 2008; Van Goozen, Slabbekoorn, Gooren, Sanders, & Cohen-Kettenis, 2002; Kruijver et al., 2000; Luders et al., 2009; Swaab, Chung, Kruijver, Hofman, & Ishunina, 2001; Swaab, 2004; Swaab, 2007; Swaab & Hofman, 1995). Research shows how the sexual organs appear well before the route of sexual differentiation of the brain during fetal development. In short, during the *intrauterine period*, the human brain will either turn in the direction of a male brain, if there is testosterone activity, or the female direction in the absence of testosterone (Swaab, 2004; Swaab, 2007). It is believed that gender identity, sexual orientation and other basic human sexual behaviors are predetermined in the human brain during this critical period (Bao & Swaab, 2011).

As suggested—biological factors appear to influence the likelihood of gender dysphoria (Kolb, Whishaw, & Teskey, 2016). Chromosomal abnormalities, atypical sex hormone levels, polymorphisms of the genes for the estrogen and androgen receptors are factors for architectural and functional differences in the hypothalamus, which match transsexual individuals' felt identities (Bao & Swaab, 2011; Kolb et al., 2016). Conclusively, differentiation in gender identity appears to arise from prenatal

events, not from social nor environmental exposure (Bao & Swaab, 2011; Kolb et al., 2016).

The research question this review paper will try to answer is following: *What are the neurobiological underpinnings of gender dysphoria?* To answer this, firstly, the paper will present genetic and hormonal aspects of gender identity and gender dysphoria. This chapter will describe genetics mechanisms of sex differentiation but also variations in genetics that may influence gender dysphoria and gender identity. Due to the limitation of space, this paper will focus especially on investigating transsexuality mainly from a neurobiological view. Furthermore, the chapter will discuss hormonal mechanisms of sex differentiation and in regards to transsexuality discuss variations in sex differentiation of hormonal activation. Second, this paper will describe the neurodevelopment of gender identity and gender dysphoria through sex differentiation in neurodevelopment where the paradigmatic hypothalamic sex differences will be specified. With this in mind, variations in sex differentiation in terms of neurodevelopment through two key areas will be provided. Finally, a comparison between gender dysphoria and DSM-5 in terms of diagnosis and research, moreover, this paper questions whether gender dysphoria should be considered a mental disorder at all. Conclusively, there is biological evidence for sex differentiation and indications that lead science into considering biological components for gender dysphoria as well. Although, future research is recommended to focus more on the possible genetic factors of gender identity in order to bring upgraded facts to the table. Furthermore, considering larger sample sizes and more replications. Incomplete knowledge of what exactly constitutes an individual's gender identity is still the reality.

Sex differences in the brain may lay the foundation not only for sex

differences in reproduction, gender identity and sexual orientation but also for differences in the prevalence of psychiatric and neurological diseases in adulthood and in age-related neurodegeneration among the sexes (Swaab et al., 2003). However, neurodegeneration or different neurological diseases are not what this paper will cover. Furthermore, although organizing effects of sex hormones operate in other parts of the brain, such as the amygdala, prefrontal cortex and spinal cord (Kolb et al., 2016), this paper will aim at the decisive region the hypothalamus.

The term *transsexual* is in many ways considered out-of-date (Blaney & Millon, 2008; Chrisler & McCreary, 2010; Polly & Nicole, 2011). Nevertheless, since *transgender* is a broad term involving the third gender, cross-dressers, agender individuals' etcetera. (Polly & Nicole, 2011), this paper will use the term *transsexuals* when describing individuals experiencing the most extreme form of incongruence between *gender identity* and physical appearance (where the desire for SRS and or hormonal treatments are necessary) and *gender dysphoria* as the broader term, a general incongruence between body and mind (not directed to a specific gender plus SRS not necessary), motivated in described literature. This paper will focus on the neuroscientific findings of gender identity and discuss the cognitive mechanisms behind gender dysphoria.

It is of importance to continue to meticulously chart subjects such as gender identity for greater knowledge of the structural dimorphic differences that can lead to a useful agenda for future human health (Swaab, 2007). The paradigmatic development of sex may have a default arrangement, but variations do emerge. These variations are what this review paper will try to illuminate. In sum, overall findings display how one's gender identity is much less about choice and more about biology (Coolidge, Thede, & Young, 2002).

2. Genetic and Hormonal Aspects of Gender Identity and Gender Dysphoria

After introducing a background on gender dysphoria, it is of importance to illustrate the nature of paradigmatic gender differentiation with regards to genetics, hormones but also examine variations that may arise due to alterations and might influence the experience of gender identity. Differentiation in biological sex in utero will be examined in this chapter through diverse paragraphs focusing on genetics and also, hormones—although these are highly dependent on each other.

2.1 Genetics mechanisms of sex differentiation

The synergy of genes and environment shape human behavior (Kandel, Schwartz, & Jessel, 1995). When it comes to the problem of sex differentiation it is no different, the typical female/male genitalia is influenced both by genetic and hormonal factors. Through genes, the encoding of proteins that are crucial for development, maintenance and homeostatic behavior is carried over to future generations. From *in utero* (in the womb) until after birth, both the genetic and developmental specifics affect our way of acting (Kandel et al., 1995; Kolb et al., 2016). While addressing behavior, the combination of what is instinctive and what is taught are often discussed (Kandel et al., 1995). Consequently, while exchanging views on instinctive behavior, usually the genetic heritage is considered. Human sexual behavior is considered an instinctive way of behaving (Kandel et al., 1995; Kolb et al., 2016).

The type of *gonad* (sex organ) that produces sex cells is decided through a single gene. The type of gonad will affect what hormones the fetus will develop. The *gonadal hormones* (also called sex hormones) to which the infant is exposed, will lead to the development of specific tissues along sexually dimorphic activity. To clarify, *sexual dimorphism* refers to the diversity of attributes in males and females

and the differential development of brain regions. In short—for the most part, the ovaries secrete estrogens, and testes androgens (Kandel et al., 1995; Kolb et al., 2016). Through conception, the sex of a human individual is established through chromosomes X or Y in the sperm (Bao & Swaab, 2011; Kandel et al., 1995; Kolb et al., 2016; Ngun et al., 2011). The most apparent genetic differences in terms of sexual differentiation between males and females are their sex chromosome counterpart (i.e. 46, XX is a typical female and 46, XY is a typical male) (Ngun et al., 2011). The embryonic gonad will, in turn, develop either testes or ovaries. This occurrence evolves in the sixth week of pregnancy (Swaab, 2004; Swaab, 2007). In addition, the sexual differentiation is regulated through the action of hormonal activation. Therefore, the sex-determining gene on the Y chromosome (the SRY) will produce testes, and consequently, the hormonal secretions will generate the development of a phenotypically male. On the contrary, the female phenotype, which includes ovaries, will be produced alongside the presence of the X chromosome. For this reason, if a gonad is absent, or if nothing but X chromosomes are available, the fetus will grow into a phenotypically female. Hence, the ovary is the human developmental default or preselected pathway. The Y chromosome from the father manages to change the preselected program with the characteristics of testes' sex hormones (Bao & Swaab, 2011; Kandel et al., 1995; Kolb et al., 2016; Smith et al., 2015; Swaab, 2007).

The academic work on unusual combinations of sex chromosomes has revealed how mammalian embryos carrying a Y chromosome develop into males, no matter how many X chromosomes they carry (Kandel et al., 1995; Meyer-Bahlburg, 2010). However, it is important to note that the Y chromosome does not carry out the sex differentiation alone—it is understood that a set of second genes acts in conjunction to create male qualities. Undoubtedly, without hormonal substances such

as testosterone, the fetus would evolve into a phenotypically female even though the Y chromosome is present (Kandel et al., 1995). In order to try to investigate the factors that influence gender identity, this paper will further discuss the sex differentiation of genetics, which may vary in order for alterations (such as gender dysphoria) to emerge.

2.1.1 Variations in genetics that may influence gender identity

As mentioned earlier, the paradigmatic female has a disposition of 46, XX in terms of genetics and a paradigmatic male have 46, XY (Ngun et al., 2011). Although, cases of alterations and an abnormal number of chromosomes in a cell has been seen to appear. A clinical term for this phenomenon is *aneuploidy* (Fernández et al., 2014a). The most common genetic disorder of sex chromosomes among men is *Klinefelter Syndrome* (also known as 47, XXY), which equals two or more X chromosomes (Ngun et al., 2011). In a *Klinefelter* (XXY) male, transsexuality was reported (Swaab, 2004). The syndrome equals chromosomal abnormalities, which often leads to sterility. In women, another variant entails the absence or an extra set of X chromosomes such as 47, XXX or *Turner syndrome* (45, X). Seen in females with *Turner Syndrome*, social difficulties may arise compared to typical females (Green & Keverne, 2000; Ngun et al., 2011). The impairments are believed to arise because of a genetic locus involved in social skills on the X chromosome since conducted data have shown results of 45, X females and 46, XY (typical) males experiencing poorer social adjustment in adolescence compared to 46, XX females (which has also led to potential ideas concerning males' predisposition of autism spectrum disorders), but also, males more often show struggle in learning social skills such as language (Ngun et al., 2011).

Furthermore, factors such as chromosomal abnormalities, *polymorphisms* (i.e.

altered phenotypes of genes) of the genes that may affect *aromatase*, (i.e. *estrogen synthase*, being enzymes which convert testosterone into estradiol, where both a masculinization of the brain may occur to female and males) have been hypothesized to increase the chances of developing gender dysphoria (Bao & Swaab, 2011; De Montellano, 2005; Fernández et al., 2014b). Further, an abnormal hormonal level during early development is also a factor for the development of gender dysphoria (discussed later in this chapter).

A strong hereditary component (62%) was estimated in a study consisting of a child and adolescent twins based on parental experiences of the concerned children (Coolidge et al., 2002; Swaab, 2004). Speculations on genes, such as cytochrome P450 (CYP)-17 that encodes aromatase, are studied in the literature. Furthermore, P450s 11B1 deficiencies are linked to *congenital adrenal hyperplasia* (CAH), where girls are exposed to high levels of testosterone *in utero* (Bao & Swaab, 2011; De Montellano, 2005; Smith et al., 2015; Swaab, 2007). This is due to the impaired synthesis of a hormone (cortisol), which is characterized by high blood pressure and further signs of androgen excess. Also, females with P450 21A2 deficiency are prenatally exposed to high levels of androgens, which give them masculinized external genitalia at birth. The gene CYP11A1 are linked to lipoid adrenal hyperplasia (a rare disorder, the lethal kind) (Kim, 2014), but also occasional CAH. Although, the gene CYP21A2 has been linked to more than 90 % of all cases of CAH (De Montellano, 2005; Swaab, 2004). Historically, gender change has been found among individuals with CAH (Meyer-Bahlburg et al., 1996; Swaab, 2004). This concludes how cytochrome P450 is highly connected to CAH development (De Montellano, 2005). Furthermore, P450 17A1 (CYP-17) as aforementioned seems to play an important role in human steroid metabolism, where mutations have been connected to

ambiguous genitals (also intersex appearance) due to then deficiency of important enzymes (such as lyase).

In another attempt to find the relation between genes, including CYP19A1 (the "classic" aromatase) (De Montellano, 2005) and transsexuality, a total of 442 MTF transsexuals and 473 XY (a chromosomal male) control males, were included (Fernández et al., 2014a). Although no association could be stated, the researchers stumbled upon a serendipitous discovery. Abnormal chromosomal numbers (the prevalence of *aneuploidy*) (2,04%) were found in the group of MTF transsexuals, which is slightly higher than the average population according to Fernández et al. (2014a). Individuals affected by *Klinefelter Syndrome* were among others in the abnormal chromosome sample.

Correspondingly, Bentz et al. (2008) investigated (CYP)-17 and a mutation in this gene (CYP17 -34 T>C SNP) was connected to female-to-male (FTM) transsexualism. Both male-to-female (MTF) (*n*= 49) and FTM (*n*= 102) groups were represented in the study, although no significance among MTF transsexuals and male controls was maintained (Bentz et al., 2008; Smith et al., 2015). Ujike et al. (2009) investigated the genetic variants of sex hormone-related genes but found nothing that could support interconnection. Moreover, there is too little information about genetic factors that might influence gender and cause gender dysphoria (Swaab, 2004). Although, theories on genomic imprinting (i.e. the epigenetic process of genes being expressed or silenced) have been discussed (Green & Keverne, 2000; Swaab, 2004). The genetic theory of male homosexuality, that implies how homosexuality tends to cluster in families varying the ratio of maternal aunts and uncles (where homosexual males found a significant deficit in the number of uncles) has led to theories on X chromosomes that may be imprinted and inherited, and perhaps applicable in cases of

transsexuality (Green & Keverne, 2000; VanderLaan & Vasey, 2011). Conclusively, to have a better understanding, more research in regard to genes and heritability is needed to provide any sufficient verification.

Furthermore, this paper will highlight the importance of hormonal activation and development of gender identity identification, both in terms of paradigmatic sex differentiation but also variations that have been shown to emerge.

2.2 Hormonal mechanisms of sex differentiation

Not unexpectedly, the brain's sexual phenotype is dependent on exposure to specific steroid hormones during a critical phase, when the nervous system is making initial progress (Kandel et al., 1995; Kolb et al., 2016). *Critical phases* (limited periods of time when the brain is sensitive to various steroids) govern the specific phases of differentiation and predict how the offspring will progress. Steroid hormones, such as the hormone *testosterone*, have reviewed effects on brain development. Rodents have been used as model systems in studying these effects. In short, during the *intrauterine period*, the human brain will either turn into the direction of a male brain, if there is testosterone activity or the female direction that is operative with the absence of testosterone (Smith, et al., 2015; Swaab, 2007). During the development of a male rat, his testes secrete *testosterone*, which will masculinize the nervous system among others. During this time, gender identity and sexual orientation are believed to be preselected in the human brain (Luders et al., 2009). The sense of self, or our maleness or femaleness, plays an important role in how we form our relationships with others but also with ourselves (Ngun et al., 2011).

Furthermore, the production of *testosterone* and other androgens (such as *dihydrotestosterone*) through the male testes is crucial for the development of male sexual organs (Kandel et al., 1995; Kolb et al., 2016; White & Porterfield, 2012). This

occurs between weeks six and twelve of pregnancy (Swaab, 2007). To clarify, the gonads in both sexes possess receptors capable of synthesizing either androgens or estrogens (Kandel et al., 1995). The products of steroids in ovaries and testes differ in their timing and proportion, hence, in some parts of their synthetic pathways. To summarize, the two pathways show how there is a sexual differentiation of the nervous system (Kandel et al., 1995; Schwarz, 2016). Conclusively, the sex differences in the developing nervous system express sex-specific behaviors in adults (Schwarz, 2016). As a consequence, the development of the nervous system can be altered by the administration of steroids. Exposure to testosterone in a female rat can have a permanent organizational effect on her nervous system (Schwarz, 2016).

Furthermore, the placenta acts as an endocrine gland (Kandel et al., 1995). During growth *in utero*, the exact role of placental secretions is not satisfactorily known though, although observation has demonstrated how the placenta from maternal cholesterol could metabolize progesterone. A hormone called *progesterone* from the placenta is sent to the adrenal glands and liver of the fetus to be altered into estrogens and androgens (inclusively testosterone). Simultaneously, the hormones return to the placenta (Kandel et al., 1995). Assuredly, the androgens needed for the emergence of the male pattern are developed through both the placenta and the developing testes. In experiments carried out on rats, it was shown that testes began to synthesize androgens early in the fetal development: in fact as early as the thirteenth day of development. The testes kept secreting androgens as late as the tenth day after birth. To detect if there is causation, newborn rats have proven the importance of androgen availability. The effects of the castration showed a great consequence on the sexual development of the genotypic rat. An exemplary female behavior was adopted among the castrated rats injected with estrogen and progesterone (for example

lordosis behavior, i.e. a reflex action causing many non-primate female mammals to make a body posture for sexual responsiveness to copulation). In addition, the male rats that were castrated after ten days of age showed only a minor proneness of the same behavior (Kandel et al., 1995; Kolb et al., 2016).

As aforementioned, *in utero*, a fetus of any sex is exposed to the high levels of distributed estrogens in the maternal blood (Kandel et al., 1995; Swaab, 2004). Kandel et al. (1995) describes a study in which when fertile, guinea pig females were injected with heterotypical hormones (e.g. androgens), alterations were aroused. The female guinea pigs were born intersex. They developed typical male external genitals but also internally had the female parts internally. When they grew into adult guinea pigs, the researchers exposed them to estrogen and progesterone, which made them develop more typical female behavior (the aforementioned *lordosis behavior*). At the same time, the guinea pigs demonstrated more mounting during copulation, which is an atypical female guinea pig behavior. The example of the guinea pigs shows how decisive hormone exposure is during development (Kandel et al., 1995).

Not just testosterone, but additionally estrogen has been seen to induce masculinization (Kandel et al., 1995; Kolb et al., 2016; Schwarz, 2016). Seen in experimental conditions with newborn rats, the sexual differentiation of the brain starts off with cells in the brain producing *aromatase* (i.e. *estrogen synthase*, being enzymes which convert testosterone into estradiol) (Kolb et al., 2016). In spite of the steroid hormone *estradiol*, which is often considered "the female hormone", has shown how after birth, estradiol could be more favorable in androgenization than testosterone. In comparison—high levels of maternal estrogen do not masculinize the female brain (Bakker & Baum, 2008; Kandel et al., 1995; Kolb et al., 2016). The aromatization hypothesis explains how both androgens and estrogens could

masculinize the brain (Schwarz, 2016). As understood, during growth *in utero*, fetuses of any sex are exposed to the high levels of estrogens in the maternal blood. However, in female rat fetuses, an estrogen-binding liver enzyme (*α-fetoprotein*) prevents the transport of estradiol to the brain so that the female rat baby is not being exposed (Kandel et al., 1995; Kolb et al., 2016; Schwarz, 2016). The enzyme is hypothesized to protect the fetus from the maternal estradiol that otherwise could have a masculinizing effect on the development of the fetus (Bakker & Baum, 2008). Chiefly, this enzyme does not bind onto testosterone, thus the hormone has entrance to steroid-sensitive neurons during the critical period (Kandel et al., 1995; Swaab, 2007). Swaab (2007) continues to argue how the female brain is protected against the effect of circulating estrogens through the maternal blood by the liver enzyme, which the fetuses produce themselves. Nevertheless, the idea of an enzyme protecting the female brain from the differentiating action of estrogens has been questioned, some think this enzyme is but a transporter and not a protector (Bakker & Baum, 2008). On another note, the aromatization hypothesis has been tested on rodents and does not automatically become facts related to the human brain. Although, it is important to consider since a lot of our knowledge regarding the underlying mechanisms of brain's sexual differentiation has developed from research in rodents (Schwarz, 2016).

Conclusively, the early influences of sex hormones during fetal development have been described. On this note, males show two periods of higher testosterone levels during early development (Swaab, 2007). Throughout mid-pregnancy, the testosterone levels peak between weeks twelve and eighteen. During the time between weeks 34 to 41, males are predicted to have at least ten times more testosterone than female fetuses during the same stage of development. Swaab, (2007) continues to describe how after birth the second peak appears. The peak in testosterone appears in

the first three months after delivery. Just before birth, the aforementioned protein, which protects the female fetus from too much estrogen exposure drops and estrogen exposure in the placenta, is a fact. This occurrence inhibits the hormonal pituitary gland, hypothalamus and gonadal glands (also called the *hypothalamus-hypophysial-gonadal axis*) but after being born the inhibition is lost. Therefore, a peak in testosterone in males occurs and in females a peak in estrogen. The levels in the male newborn at this time can be compared to adult men, although a lot of it has no alternative but to circulate (Swaab, 2007). No comparison of the same testosterone levels is found during the process of the female. Swaab (2007) argues how the programming of structures and circuits in the male brain occurs during these peaks of testosterone. Circumstances that could prevent the communication of these hormones in the brain, can permanently affect later behavior in life (Swaab, 2007).

For the most part, sexually dissimilar brains often practice divergent behavioral tendencies (Kandel et al., 1995; Kolb et al., 2016). Genetically female, prepubertal rhesus monkeys exposed to sources of androgens showed a more aggressive and less maternal behavior, compared to other monkeys of the same sex and age. Animals who were androgen-exposed during the early critical period played with other monkeys exposed to similar hormonal levels. The genetic sex of the new friend did not seem so important to them (Kandel et al., 1995).

Among humans, girls affected by the aforementioned CAH have had their behavior investigated as well. Some studies show how the affected girls tend to prefer to play with boys, more often choose the ”boys’ toys” and are roughly wilder than the majority of girls in their behavior (Swaab, 2007). Also, CAH inflicted girls frequently show male characteristics in puberty (such as abnormal or absent periods, a deep voice, early puberty and facial hair). Studies have shown how these girls in a higher

degree turn out to be homosexual or transsexual (Bao & Swaab, 2011; Berg-Weger, 2016; Chung et al., 2002; Swaab, 2007).

Because of the external and internal genitals being constructed earlier in the evolution of the fetus, the sexual differentiation of the brain may be influenced independently. This may result in individuals with male sexual organs, experiencing themselves as females (the reality of transsexuals). It is important to highlight how the hormonal and developmental action during the perinatal critical period of sexual differentiation of the nervous system does not result in an absolute feminization or masculinization of the brain (Kandel et al., 1995; Swaab, 2007). Different degrees of normative and atypical progress are normal and part of the individual differences among natural variation. Particularly, human beings possess different levels of estrogen and testosterone (Kandel et al., 1995). In conclusion, the level of feminization or masculinization of the genitals may not display the same level of the feminization or masculinization of the brain (Kandel et al., 1995; Swaab, 2007).

2.2.1 Variations in sex differentiation of hormonal activation

Abnormal levels of early hormones may also increase the chance of developing gender dysphoria such as CAH where girls are exposed to high levels of testosterone *in utero* (in the womb) possess these characterizations (Bao & Swaab, 2011). Further, a risk of inheritance of among Caucasians is equivalent to one in 5000-15000 newborns, and a large variability among different population groups. CAH could be described as an autosomal, recessive group of disorders amongst steroid hormone synthesis (Zucker et al., 1996). Furthermore, Hughes (2008) sustains how CAH is the most common cause of ambiguous genitalia among babies. CAH may be correlated with transsexuality, but with moderate results (5.2% probability of serious gender problems) Bao and Swaab, (2011) concludes. Some chemicals in

medications given to pregnant mothers, so called *phenobarbital* and *diphantoin* (for epilepsy and seizures) have shown a larger possibility of giving birth to transsexual children. It is believed to change the metabolism of the sex hormones and in turn, could act on the sexual dimorphism of the fetus' brain (Bao & Swaab, 2011).

A phenomenon called hormonal resistance occurs when a specific hormonal activity is inactive due to receptor loss or inactivation (Kandel et al., 1995; Swaab, 2004; White & Porterfield, 2012). The phenomenon of *androgen insensitivity syndrome* (AIS) shows how affected individuals possess a set of XY chromosomes thus the secretion of testosterone—although, since the individuals cannot be compatible with the androgens, they look and develop female traits because it will have no effect on estrogen receptors (Kandel et al., 1995). Consequently, the fetus will further develop the form of the uterine tubes and the upper portion of the vagina except for no uterus or oviducts (Kandel et al., 1995). Gender identity can evolve although hormonal influences are absent from the fetal gonads. Therefore, the consideration of gonadal hormones domination on the maturing and growing nervous system is a pivotal first step (Kandel et al., 1995; Swaab, 2007). As mentioned, AIS in alternative forms equals clinical syndromes of some hormonal resistance. Side effects, such as atypical genitalia of the baby or some defects in testis determination are caused by androgens working in an atypical manner. Hughes and Deeb (2006) continue to describe how altered mutations of the long arm of the X chromosome can result in different androgen receptor problems. Dysfunctions make sex assignment and psychological guidance more common among the affected individuals (Hughes & Deeb, 2006; Quigley et al., 1995).

Additionally, there are different categories when addressing the subject of AIS: *Complete androgen insensitivity syndrome* (CAIS) is equivalent to what the

name suggests: a comprehensive inability of the cell to be able to act upon androgens. Testosterone plays a cardinal role in terms of gender identity and sexual orientation, which has been identified through numerous disorders (Bao & Swaab, 2011; Cohen-Kettenis, 2005; Swaab, 2004). Hence, individuals affected by CAIS (XY individuals) are born with female genitalia (Hughes & Deeb, 2006). Moreover, the reasons why CAIS occur are several mutations of the accountable gene, which cause males affected by CAIS to develop into phenotypically females.

With a resemblance to CAIS, 5α-reductase-2 or 17β-hydroxysteroid dehydrogenase-3 deficiency is caused by the failure of androgen conversion (Bao & Swaab, 2011; Cohen-Kettenis, 2005; Swaab, 2004). A male fetus could have had problems with sufficient testosterone transformation and the XY-children could develop into girls with a large clitoris (although, on the inside having male genitals). Customarily, these children are brought up as girls (Cohen-Kettenis, 2005). Although, different to CAIS in a sense of testosterone levels increasing in puberty, thus, the ”clitoris” is able to grow into a regular sized penis and the hidden testicles become lowered. With the growing muscles due to testosterone and more typical male features, the majority of these nurtured girls subsequently adopt a new identity as heterosexual males due to the masculinization at puberty (Bao & Swaab, 2011; Cohen-Kettenis, 2005; Swaab, 2004).

On this note, Swaab (2004) argues how the male patients with 5α-reductase-2 or 17β-hydroxysteroid dehydrogenase-3 deficiency indicate how testosterone during development and at puberty seems to have a more important impact in determining male gender identity than of rearing and of sociocultural influences. Cohen-Kettenis (2005) further points out that these findings raise further doubt regarding the theory of children being gender-neutral at birth.

Another category of AIS, *Mild androgen insensitivity syndrome* (MAIS), affects men born with male genitalia. This category of AIS was found while investigations of male infertility and suggested a defect in androgen action, except for a less degree that AIS but still enough to be able to manifest itself in some cases of gynecomastia (an increase in size of male breasts) and abnormalities of the urethra on the male penis, when the usual location of the unitary opening is not placed on the head of the penis. *Partial androgen insensitivity syndrome* (PAIS) is one cause of intersex (Hughes, 2008; Hughes & Deeb, 2006; Quigley et al., 1995). A PAIS phenotype is commonly affected with micropenis, a scrotum or as mentioned above, abnormalities of the unitary opening placement. PAIS phenotype has a partial insensitivity to male sex hormones compared to phenotypically males. Minto, Liao, Woodhouse, Ransley & Creighton (2003) sustain how approximately one in 2000 births are born intersex.

From the information covered so far, one could understand how many steps during the process of sex development that could be disrupted due to alterations. Therefore, the theory of the two processes of sexual differentiation of the genitals and of the brain which could be influenced independently of each other, seems to have had great influence on present-time enquiries (Bao & Swaab, 2011; Berglund et al., 2008; Cohen-Kettenis & Gooren, 1999; Chung et al., 2002; Garcia-Falgueras & Swaab, 2008; Van Goozen et al., 2002; Kruijver et al., 2000; Luders et al., 2009; Swaab et al., 2001; Swaab, 2004; Swaab, 2007; Swaab & Hofman, 1995).

Bao and Swaab, (2011) further conclude how no evidence has been provided that satisfactorily presents how an individual's postnatal social environment is definite in terms of gender identity. An example was found among newborn male babies born with severe birth defects of the lower abdominal organs (e.g. cases of

bladder exstrophy-epispadias-cloacal exstrophy complex, a spectrum of anomalies involving the urinary tract, genital tract and musculoskeletal system), where parts of the penis or the whole penis could be missing. Commonly, males with bladder exstrophy and cloacal exstrophy become corrected into females shortly after birth (Bao & Swaab, 2011; Swaab, 2004). When the group of people who had experienced gender dysphoria within this calculation was excluded, only 47% was well off with their gender identity. This again, supports the idea of an intrauterine period of programming gender identity in the brain, rather than only through socialization or learning-dependent contexts (Bao & Swaab, 2011). In cases of males being gender-reassigned as females where gender identity has been questioned by the patients themselves, subsidize the theory of early programming of gender identity by biological factors (Swaab, 2004). Hence, the lack of support for social factors supports the theory of gender identity into being largely a biological phenomenon. Furthermore, this paper will continue to examine the neurobiology of gender identity and gender dysphoria through the understandings of brain organization and neurodevelopment.

3. Neurodevelopment of Gender Identity and Gender Dysphoria

After introducing a background on the genetic and hormonal aspects of gender identity and gender dysphoria, a chapter on neurodevelopment and brain organization of gender identity and gender dysphoria will be provided. Firstly, this will be discussed through sex differentiation in neurodevelopment and paradigmatic hypothalamic sex differences. Second, variations in neurodevelopment with emphasis on two important hypothalamic structures will be offered.

3.1 Sex differentiation in neurodevelopment

The understandings of human brain development have changed over the years. During the 1960's and 1970's, it was an accepted fact that a child was born as a *tabula rasa* i.e. a clean slate. Thus, the formation of gender identity was formed entirely by society's norms and expectations (Bao & Swaab, 2011; Swaab, 2004; Swaab, 2007). John Money, psychologist and researcher specializing in the subjects of sexual identity and biology of gender, made a well-known experiment known as the twin study of the "John/Joan" case where a botched circumcision left an eight-months-old boy with no penis. According to the hypothesis of Money—gender imprinting did not start until the age of one, therefore, his testicles were removed and the decision to turn the baby into a girl was settled. Although, the dressing of the child in girl's clothes, counseling during upbringing and estrogens given in puberty did not change the fact that this individual changed his gender back to male as an adult. The man even got married and adopted children, but tragically committed suicide in 2004. The importance of the intrauterine period on gender is illustrated in this story (Bao & Swaab, 2011; Swaab, 2004; Swaab, 2007). Furthermore, gender identity has been researched for years but has not always been taken seriously (Bao & Swaab, 2011; Cohen-Kettenis & Gooren, 1999; Luders et al., 2009). Early on, societal susceptibility

to parental and family factors was held responsible for children who developed gender dysphoria, as the absence of a father, extreme attachment to the mother, or a maternal wish for a daughter.

Linked with the previous paragraph on sex neutrality at birth, no robust empirical studies have supported these hypotheses either to be true. Hence, there has been no indication of postnatal social factors that would be responsible for transsexuality (Bao & Swaab, 2011; Cohen-Kettenis & Gooren, 1999; Kolb et al., 2016; Swaab, 2004). In the scientific literature, transsexualism is considered a disorder with multifactorial etiology (Fernández et al., 2014b). Factors that correlate with it include, but are not necessarily limited to: genetic factors, hormonal effects, differences in brain anatomy and with a particular emphasis of the hypothalamus, all discussed further in this paper.

Bao and Swaab (2011), argue that from the beginning of life, babies already express sex differences in behavior. After being born, female infants gaze more at human faces that male infants. At the same time, male neonates prefer to look more at mechanical mobiles (Bao & Swaab, 2011). Furthermore, later research involved play among children (Alexander & Hines, 2002; Bao & Swaab, 2011; Swaab, 2007). Sex differences in toy inclination among children are thought by many to arise from gender socialization and social pressure. Alexander and Hines (2002) study on vervet monkeys showed how biological factors during early development (e.g. levels of androgens) could influence the choice of toys. In female vervets, the percent of contact time with toys preferred by human boys (in this case, a ball and a car) was less than in male vervets and vice versa (toys preferred by human girls was introduced to male and female vervets). Neutral toys (a stuffed dog and picture book) had equal contact time amidst the sexes. The different preference of features such as color,

shapes and movements is hypothesized to have had evolutionary advantages in the development of a hominid lineage, moreover, that the different behavior roles assigned might contribute to present day in dimorphic preferences in toys (Alexander & Hines, 2002).

Such sexually dimorphic differences in the neuronal organization have been established due to adaptations in the growth rate of dendrites and axons between the sexes (Kandel et al., 1995). As aforementioned, the steroid sex hormones during critical periods might prejudice the rate of axonal differentiation in various regions in the brain. This leads to an effect on neuronal circuitry and a rivalry for postsynaptic settings between populations of axons of different origins. To clarify, this could produce sex differences in neural circuitry (Kandel et al., 1995).

Although, sexual differentiation of the brain is believed not only to be affected by the hormonal activation: Swaab (2007) continues to describe how there are many additional genes participating in the sexual differentiation of the brain. Not only hormones are important to study in terms of sexual differentiation of the brain, but also, the cooperation and interplay between genes and hormones.

Several sex differences are thought to reside in the hypothalamus, such as reproduction, sexual orientation and the identification of gender but also menstrual cycles in women, prevalence of psychiatric and neurological diseases—however, the three last assertions will not be discussed further (Allen & Gorski, 1990; Byne et al., 2000; Swaab, 2007; Swaab et al., 2001; Swaab et al., 2003; Swaab & Hofman, 1988; Swaab & Hofman, 1995).

3.1.1 Paradigmatic hypothalamic sex differences

Numerous regions of the hypothalamus is recognized as being sexually dimorphic structures (such as *the anterior commissure* (AC), *darkly staining*

posteromedial component of the bed nucleus of the stria terminalis (BNST-DSPM), *the central nucleus of the bed nucleus of the stria terminalis* (BSTc), *the interstitial nucleus of the anterior hypothalamus* (INAH) -2, 3, 4, *the suprachiasmatic nucleus* (SCN), and *sexually dimorphic nucleus of the preoptic area*, acronymized SDN) (Allen & Gorski, 1990; Bao & Swaab, 2011; Ngun et al., 2011; Swaab, 2007; Swaab et al., 2001; Swaab et al., 2003; Zhou, Hofman, Gooren, & Swaab, 1995).

In mammals, the cell nucleus of the cells in the medial preoptic area and in the hypothalamic forebrain is considerably smaller in females' volume of these regions while compared to equal parts in the males (Kandel et al., 1995). This part of the brain has been called the *sexually dimorphic nucleus of the preoptic area* (SDN-POA). Tentatively, the four different INAH structures of the hypothalamus have been considered as possible human nuclei for homologous relationships' when compared to the rat's SDN-POA (Byne et al., 2000; Swaab et al., 2001). Swaab et al. (2003) have argued that the SDN-POA in the rat is highly similar to the INAH-1 in humans. In addition, another part of the hypothalamus called the INAH-3 is engaged in sex differences analogous to language and cognitive abilities and handles the left-right connections of the temporal cortex. This region is larger in female brains than in male brains (Bao & Swaab, 2011). Allen & Gorski, (1990) described another hypothalamic structure (the BNST-DSPM) that had a volume of 2.5 times larger in human males compared to females. Brains of 26 human subjects were used and studied. Also, the BSTc was observed to be 40% larger in men (also twice as many neurons) when compared to the women of the study (Kruijver et al., 2000; Swaab et al., 2001; Zhou et al., 1995; White & Porterfield, 2012). Further information from this study will be provided later in this paper.

Sex differences are also believed to differentiate in terms of preferences in

sexual orientation and are of important to identify in terms of gender identity and transsexuality. Swaab (2007) emphasize the importance of activity of the hypothalamus in terms of sexual orientation. The activity of the serotonergic system has been examined and through studies on the influence of pheromones, similarities between a heterosexual woman and homosexual men have been demonstrated (Savic, Berglund, & Lindström, 2005; Swaab, 2007). The study showed how heterosexual males showed no effect from being stimulated with a male scent, although in the hypothalamus of a heterosexual woman and a homosexual man, that response was found. Hence, pheromones have been hypothesized to play a role in sexual orientation. Furthermore, because of the close association between regions in the brain (which seem to be responsible for sexual orientation and other parts responsible for the sense of gender identity), the importance of taking the aspect of sexual orientation into consideration whilst addressing brain differences in transgender individuals is inevitable.

While trying to define alterations in brain organization—it is unwise to confuse sexual orientation and gender identity to be the same thing because as mentioned earlier, transsexualism being independent of sexual orientation (American Psychiatric Association, 2013; Cohen-Kettenis & Gooren, 1999; Swaab, 2007). This chapter will continue to examine variations in brain organization, which may influence gender identity and furthermore, gender dysphoria.

3.2 Variations in neurodevelopmental sex differentiation

A theory on the development of gender dysphoria and furthermore, transsexuality development resulted from the fact that sexual differentiation of the brain and differentiation of sexual organs develops during different time periods. Many studies sustain how the sexual organs appear well before the route of sexual

differentiation of the brain in fetal development (Bao & Swaab, 2011; Berglund et al., 2008; Cohen-Kettenis & Gooren, 1999; Chung et al., 2002; Garcia-Falgueras & Swaab, 2008; Van Goozen et al., 2002; Kruijver et al., 2000; Luders et al., 2009; Smith et al., 2015; Swaab et al., 2001; Swaab, 2004; Swaab, 2007; Swaab & Hofman, 1995). Luders et al. (2009) investigated this theory with magnetic resonance imaging (MRI) data. A group of MTF transsexuals (*n*= 24) that had not yet been exposed to any hormonal treatment were analyzed together with control women (*n*= 30) and control men (*n*= 30) to determine whether MTF transsexuals held a brain more comparable to their *natal* (born with) gender or more adjacent to individuals who share the same gender identity. This was measured by studying the participants grey matter. The study discovered significant dissimilarities in MTF transsexuals, males and females in a large number of regions across the brain. The overall MRI results indicated that men and MTF transsexuals regional grey matter volumes had significantly more grey matter than males, which is more comparable to females grey matter which showed a significant result in having the most grey matter in right *putamen*. Conclusively, a significantly larger volume of regional grey matter in the right putamen was discovered in MTF transsexuals, compared to control men, which suggests a ”feminization” of the brain. Furthermore, *putamen* constitutes with *globus pallidus* and *caudate nucleus* the region *basal ganglia* (Kolb et al., 2016). Moreover, research implicates that basal ganglia primarily is responsible for action selection. This detection would be an indication of modifications in brain anatomy to be involved in gender identity. However, the study did not draw any conclusions to what exact behavior this might affect, thus calls for future research. A limitation of this study was how MTF transsexuals were measured as one homogenous group concerning sexual orientation. From what we have examined earlier, transsexuality is

independent of sexual orientation and therefore should be controlled (American Psychiatric Association, 2013; Cohen-Kettenis & Gooren, 1999; Luders et al., 2009; Swaab, 2007). The result of the MRI study was coherent with a study in positron emission tomography (PET), presented by Berglund et al. (2008). In this study, sexual orientation was controlled for. The activation condition consisted of passive smelling of two synthetical steroids (a substance present in human male secretions and another derived from the urine of pregnant women). According to the researchers, these substances could allegedly influence physiological arousal and context-dependent mood of the participants. A group of MTF transsexuals (*n*= 12) who had no hormonal treatment showed different results in the left putamen whilst exposed to odorous steroids stimulus compared to a group of control men (*n*= 12). Furthermore, group analyses showed how MTF transsexuals differed only from the male controls (Berglund et al., 2008).

In addition, parts of the hypothalamus are understood to be imperative in terms of gender identity and sexually dimorphic behavior (Kolb et al., 2016). The hypothalamus is a small but important portion of the brain and is located along the brain's midline and below the thalamus in each hemisphere (Kolb et al., 2016). Hypothalamus is decisive in many parts of human behavior. Although constituting only 0.3% of the brain's total weight, hypothalamus plays a role in some parts of sleeping, feeding, temperature regulation, emotional and sexual behavior, hormone function and movement. Kolb et al. (2016) further claim how hypothalamus is composed of 22 small nuclei and all of the related nerve fibre systems. Many of the hypothalamic nuclei are sexually dimorphic and are believed to be active in roles of sexual behavior and gender identity (Kolb et al., 2016).

Evidence has further sustained how the BSTc of the hypothalamus is assumed

to be decisive in terms of transsexuality (Bao & Swaab, 2011; Chung et al., 2002; Kruijver et al., 2000; Zhou et al., 1995). A female-sized BSTc was found in MTF transsexuals, hence, this finding supports the hypothesis of transsexuals' brain structure to be different from their external sex appearance (Kruijver et al., 2000; Swaab, 2004; Swaab et al., 2001). Consequently, brain and genitals may develop in opposite directions and this furthermore points to a neurobiological basis of gender identity (Bao & Swaab, 2011; Swaab, 2004; Kruijver et al., 2000). The observations could not be explained due to differences in adult hormonal levels, since the study controlled for this variable through non-transsexual male participants that had removed one or both of their testes (orchiectomies) but also transsexual participants who had gone through the same procedure, where the non-transsexuals men showed results in the normal male range.

Unquestionably, an important remark is that a change in BSTc does not become evident until early adulthood; thus, one cannot use this region for early diagnosis of transsexuality (Bao & Swaab, 2011; Swaab, 2004). The late occurrence of sexual dimorphism of this brain region and the often-early occurrence of gender identification in transsexual individuals make this brain region not sufficient in explaining the enigma of transsexualism (Swaab et al., 2003). This confusion leads researchers to believe there has to be more to the conundrum. Since the variable of differences in adult hormonal levels was controlled for, this was not discussed further. Tentatively, Swaab (2004) suggest the inconsistency between the late manifestation in the BSTc and often, early experience of gender dysphoria could possibly be due to functional differences in the BSTc which might herald before the structural sex differences during neurodevelopment. However, further research on this issue is required. A more profound discussion on the BSTc in regards to transsexuality is

provided later in this paper.

Other conclusions established in another part of the hypothalamus called the INAH-3, maintain the idea of a development of sexual differentiation of the brain and differentiation of sexual organs during different time periods (Garcia-Falgueras & Swaab, 2008). Furthermore, Swaab and Garcia-Falgueras (2009) conclude how differences in the human INAH-3 and BSTc supports the theory on two processes developing at different timetables to be consistent with the theory on transsexuals who has developed variations from the paradigmatic sex differentiation.

Additionally, a diffusion tensor imaging (DTI) study conducted by Rametti et al. (2011) found that the white matter patterns in hormonally untreated MTF transsexuals (*n*= 18) showed results in between results of female (*n*= 19) and male controls (*n*= 19). The purpose of the study was to see if the white matter patterns among MTF transsexuals before hormonal treatment are more analogous to those with their natal sex or those of their gender identity. Pathology in the endocrine system was controlled for and not found in the group of MTF transsexuals. Moreover, these sorts of results suggest how masculinization in MTF transsexuals during brain development is altered, independent on hormonal treatment (Rametti et al., 2011). In order to extend these findings, the future needs to show comparisons with hormone treated transsexuals as well to further confirmation (Rametti et al., 2011). As suggested, the hypothalamus has sexually dimorphic structures and is important in addressing sex differentiation in neurodevelopment. Additionally, this paper will put emphasis especially on two specific structures that have shown profound potency to be of importance for an individual's gender identity.

3.2.1 Interstitial nucleus of the anterior hypothalamus

The uncinate nucleus is a small, oval cluster of packed medium and small-

sized neurons (Garcia-Falgueras & Swaab, 2008). Moreover, the uncinate nucleus is found in the preoptic area of the human hypothalamus. This nucleus is composed of two subnuclei, also called INAH-3 and INAH-4. As mentioned earlier, the four different INAH has been considered as possible human nuclei for homology whilst compared to the rat's SDN-POA (Byne et al., 2000).

In a study conducted by Byne et al. (2000), they put this theory to test. The goal was to examine all four regions of INAH in humans to find a variation in volume, neuronal number and neuronal size. Human *post-mortem* (after death) brain material was assessed, controlled for pathology in neither hypothalamic regions nor infection with *the human immunodeficiency virus*—HIV. To reduce experimental effects there was no knowledge on the sexual orientation of the included subjects (Byne et al., 2000). The result showed how INAH-3 was smaller in females (n= 18) than in males (n= 15). The increasing volume in INAH-3 was significantly dependent on the number of neurons in the nucleus in more than 50% (p <0.05). Furthermore, SDN-POA and INAH-3 have resemblances in its sexual dimorphism (Byne et al., 2000).

Apart from differences between the sexes, an additional study conducted by Garcia-Falgueras and Swaab (2008) found a strong relationship between gender identity and parts of the hypothalamic uncinate nucleus. Moreover, with human *post-mortem* brain material (n= 42) whereas 14 control males, 11 control females, 11 male-to-female (MTF) transsexual individuals, one female-to-male (FTM) transsexual individual and five non-transsexual castrated men were participants of the study. The castrated men were so because of the diagnosis of prostate cancer (Garcia-Falgueras & Swaab, 2008).

Accordingly, the most striking dissimilarities were found in the subnucleus of

INAH-3. With a significant result (p <0.002), the volume in males contained 2.3 times as many cells than in females' INAH-3. Furthermore, for the first time, the study showed that INAH-3 volume and number of neurons was comparable between female subjects and MTF transsexual subjects (Garcia-Falgueras & Swaab, 2008). A comparison of MTF transsexual subjects and control males showed no significant result in neuronal density (p >0.063), albeit, they had a lower number of neurons whilst compared to male controls (p <.002). The researchers controlled for the confounding variable of testosterone levels through measuring the castrated group of men and found that they had an INAH-3 volume and neuron number result that was in between that of female and male controls. Due to some effect on castrated males' intermediate result, a part of hormonal levels can explain observed dissimilarities, however, not all (Garcia-Falgueras & Swaab, 2008). The authors further propose how this region in the brain is hypothesized to be decisive concerning gender identity.

In addition, the FTM transsexual individual had volume and number of neurons in the range of the male controls results. To reduce the confounding variables of estrogen treatment effects in adulthood, pre- and post- menopausal females were observed. No difference in INAH-3 was found amongst these female subjects, either in volume (p >0.84) or in a number of neurons (p <0.439).

Comparatively, brain weight among the different groups was measured Garcia-Falgueras & Swaab, 2008). Brain weight among men was higher than among female's brain weight (p <.001). When male, female, transsexual MTF and castrated patients were measured, a significant result was showed (Kruskal-Wallis p <0.008). Also, MTF transsexual subject's brain weight was in between of the males and females subjects.

However, as Byne et al. (2001) have suggested in previous studies on INAH-

3, the volume of this area might be related to the sexual orientation as well. All things considered, the data from this study disclose how the number of neurons in INAH-3 might be dependent on gender identification which is supported through data of transsexual individuals that had results that showed more similarities amongst the sex they claim they experience themselves as, rather than their born, genetic sex (Garcia-Falgueras & Swaab, 2008). Further, this finding is in contrast to earlier findings by Zhou et al. (1995), which showed how volume and neuron number, together, was related only to gender identity and not to sexual orientation (through reversed results in transsexual men but not in men who experienced themselves as homosexual). In the study conducted by Garcia-Falgueras and Swaab (2008) the researchers hypothesize how both BSTc and INAH-3 might be a part of a multifaceted neuronal network that would be functionally and structurally linked to gender identity and sexual orientation.

3.2.2 The central nucleus of the bed nucleus of the stria terminalis

The aforementioned BSTc region of the brain has been showed to be crucial in sexual behavior (Zhou et al., 1995). In animal tests, the bed nucleus of the stria terminalis (BST) has been shown to play an essential part in sexual behavior among rodents. A major centre for aromatization was found in BST of the rat's brain, but also, androgen and estrogen receptors (Zhou et al., 1995). Moreover, studies have shown how the size and cell number of the BST are sexually dimorphic in rodents but also in humans, additionally, larger in males than females. Mentioned earlier, in humans, the posterior part of the BST (BNST-DSPM) was noted to be 2.5 times bigger in men than in women (Allen & Gorski, 1990). In studies conducted on rats, masculine sexual behavior and regulation of gonadotropin release are dependent on BST activity. Hence, many reasons advocate for the BST to be of importance for

sexually dimorphic management.

Zhou et al. (1995) decided to look further at this part of the brain in humans. *Postmortem* brain material from six MTF transsexuals was collected and studied. The sexual orientation of the participants of the study was controlled for. Additionally, to control for the confounding variable of lack of androgens in the MTF transsexual subjects, two non-transsexual, castrated males (due to prostate cancer) were also participating in the study.

Results showed how the BSTc in the MTF transsexual group was female-sized. The BSTc of the castrated men were in the typical male range. One of the subjects in the MTF transsexual group had not been castrated, but nevertheless had a BSTc of a size that ranged in the middle of the transsexual group's size (Swaab, 2004; Zhou et al., 1995). In addition, since all of the participants in the transsexual group of participants had been treated with estrogens, the experimenters had to take into reconsideration the confounding variable of estrogen in the blood (although, one of the participants had stopped taking estrogens 15 months before her death because of cancer) (Zhou et al., 1995). Also, the authors describe how they had seen a 31-year-old man suffering from an adrenal tumor—which causes high levels of estrogen in the blood stream, who had a normal range male BSTc (Zhou et al., 1995). *Acquired immunodeficiency syndrome* (AIDS) was controlled for in this study, due to how the fact how AIDS might inflict specific patterns of brain damage, however, the AIDS did not seem to affect the BSTc volume or cell number (Zhou et al., 1995).

To clarify, the study presented results that showed how the BSTc part of the brain was involved in gender identity and not sexual orientation since there was no statistically significant result between heterosexual and homosexual men (Swaab, 2004; Zhou et al., 1995). Also, to exclude the confounding variable of age, they

compared the groups but no significant result was made. Furthermore, this was the very first study to manifest a female brain structure in genetically male transsexuals (Swaab, 2004; Zhou et al., 1995).

To further concretize, this outcome provides for the theory on that gender identity modifications could be a result of a modified interaction between the development of the brain and gonadal hormones (Zhou et al., 1995). Further, other examinations also settle how BSTc could be important in terms of transsexuality.

Also, differences related to gender identity during brain development were given clear support from a study conducted by Kruijver et al. (2000). In this study, the sexual orientation was regarded but also matched for age and postmortem time. Building on foundational research by Zhou et al. (1995) a total of 42 brains of patients were analyzed in the number of neurons in the BSTc (Kruijver et al., 2000). Sex hormone disorder cases, including individuals with Turner's syndrome was also present (*n*= 6), MTF transsexuals (*n*= 6), homosexuals (*n*= 9), control men (*n*= 9), but also control women (*n*= 10). Furthermore, the study had the first collected brain ever of an FTM transsexual and found innovative results of a neuronal number of the FTM transsexual participant to be in the male range. Although, problematic since due to the small sample size of FTM transsexuals, the result remained interesting. In addition, one male with gender identity sensations, yet still untreated was present in the study too.

In essence, sex reversal results in the transsexual brain through counting number of neurons were found in this study as well. Compared to heterosexual men, MTF transsexuals had 40 % lower numbers of neurons, and in comparison to homosexual males, they had 44% lower results. The BSTc was, therefore, more comparable to the female BSTc. In this study, AIDS was controlled for as well

(Kruijver et al., 2000; Swaab et al., 2003). Moreover, the cause of death, brain weight and *post-mortem* time was also taken into account but showed no relationship within group analyses. In conclusion, hormonal changes (in adult years), estrogen treatment, orchiectomy or other medical treatments did not show enough support in order to show any clear relationship with the neuron number of the BSTc (Kruijver et al., 2000; Swaab et al., 2003).

Furthermore, a study by Chung, et al. (2002) continues to support the theory of size in BSTc being important in regard to transsexuality and gender identification. Human brain tissue from 50 subjects, including children (ranging from 3 months to 16 years of age), human fetuses of both male and female sex (ranging from week 25 of pregnancy to week 41) but also adults (ranging from 22 years to 49) was obtained in order to compare the sexually dimorphic structure. The sex of the participants was 50/50 females (n= 25) and males (n= 25).

The results showed that the average was 39% bigger in males than in females (p <0.001) and therefore confirmed the previous studies that in turn have shown that the BSTc size is larger in males than in females (Kruijver et al., 2000; Zhou et al., 1995). Although, the sex difference was age dependent since the results showed how BSTc volume only showed a significant sex difference in adulthood, which shows how BSTc volume and neuron number develop much later than expected (Chung et al., 2002). The researchers concluded how sex-dependent organizational alterations in brain development of the BSTc did not seem to be limited to early development but also extend into adulthood. Furthermore, as aforementioned the late occurrence of sex differences in the BSTc and the often-early occurrence of gender dysphoria sensations make this brain region not sufficient in explaining the enigma of transsexualism (Swaab et al., 2003). Chung et al. (2002) describe how in a rat, the sexual

differentiation of the BST occurs in week one; the researchers hypothesized how this would be somewhat comparable in human fetal and neonatal development. However, since adult testosterone levels have shown no clear effects on the volume and neuron number of the BSTc (Kruijver et al., 2000; Zhou et al., 1995), it is still unclear when and how the network (or organization) of gender identity is operational. Chung et al. (2002) continue to discuss how the late sexual differentiation of other parts of the hypothalamus (such as the BNST-DSPM) does not become evident until puberty. The same has been shown to be true in (post) adolescent pigs' hypothalamus. This result does not rule out the theory on early sex hormones effects on BSTc functions, alterations in fetal or neonatal testosterone levels could affect the development of gender identity but not with immediate results (such as the masculinization that occurs in puberty, although, predetermined during fetal development). Additional neuroscientific research on the BSTc in relations to gender identity is required in order to draw any conclusions.

In sum, the analyses of INAH-3 and BSTc have pointed out structural differences in sexually dimorphic areas of the brain between control and transsexual subjects. The offered data support the theory of transsexualism having biological underpinnings. However, the results needs replication and the small sample sized are debatable (Smith et al., 2015).

4. Discussion

A dichotomy between two sexes has been one of the endless truths among human beings (Gooren, 2006). Throughout history, man and woman have been declared opposites and thus shall live as the assigned sex by the appearance of their external genitalia. Meyer-Bahlburg, (2010) describes how historically, neutrality of gender identity at birth was assumed, and thus, gender assignment was based primarily on the surgical result and/or how the genitalia came across at birth.

Furthermore, Gooren (2006) states how the definition of man and woman have led to an overall acceptance of femininity and masculinity—as not shaped by sociopsychological factors, but a product of a biological lot. The author further suggests how this acceptance may have sprung from the animal kingdom and the botanic world where animals and plants are either female or male. Somehow, it is believed that this may have had an imprint on manhood and womanhood; thus, they could be described as expressions of a natural order (Gooren, 2006). The argumentation seems plausible since preprogrammed behavior would be more time-effective than pedagogic effort from parenting to raise baby boys to be men and girls to be women. Nevertheless, the exclusivity of two genders—male or female has been questioned (Gooren, 2006). From an evolutionary perspective—is it possible that nature would not create any natural variations of these paradigmatic gender divisions?

Alternatives, such as transsexuality, homosexuality or other forms of detours of biological determinism have led to new thoughts on the subject. Meyer-Bahlburg, (2010) examines the fact that gender identity variants categorize as a mental disorder at all. The author challenges the acceptance of gender dysphoria to be a pathological condition and reasons if gender dysphoria might be a natural variation that has sprung out of evolution. Moreover, Meyer-Bahlburg, (2010) discusses how gender dysphoria

could be considered a *disorder of sex development* (DSD) instead of a mental disorder. Hughes, (2008) recommended DSD as a collection term for problems noted on external genitalia at birth, so-called *intersex* appearance, further, the definition of DSD is a congenital condition where the chromosomal, gonadal or anatomical development is unusual compared to paradigmatic gender differentiation. Hughes, (2008) hypothesizes how gender dysphoria could be defined as a DSD limited to the central nervous system—thus, without the connection of reproductive tracts. Whilst comparing the definition of gender dysphoria (see appendix) to the definition of DSD (see above), one understands how this argumentation is relevant. Furthermore, Meyer-Bahlburg (2010) reasons how the definition of DSD would be preferable and add how the psychological (gender identity) sex are incongruent—because of the seemed predetermined gender identity during early development under the influence of genetics and/or interaction with sex hormones. However, a recent web-based survey conducted results of negative feelings against the nomenclature of DSD (Johnson et al., 2017). Both individuals affected and parents had negative opinions due to causing harmful self-image of affected individuals, as suggested by the study, terms such as "genital difference or variation" would be preferable (Johnson et al., 2017).

The argumentation is based on the demonstration of MTF and FTM transsexuals reversed results in brain volume and cell number of BSTc, the INAH-3 volume and cell number and the grey matter in the right putamen (Byne et al., 2000; Garcia-Falgueras & Swaab, 2008; Kruijver et al., 2000; Luders et al., 2009; Smith et al., 2015; Zhou et al., 1995). The gist of this argumentation could be summarized as a question: should dysphoria be considered a mental disorder at all? Based on all the biological and neuroscientific findings it would make more sense for being a diagnosis of DSD—or perhaps, not a diagnosis at all considering the issues of

pathologizing questions of identity (Johnson et al., 2017).

Homosexuality, which has for many years been listed as treatable and an illness: many considered the debunking of this accepted "fact", as an act of justice and freedom. Incredibly, from one day to another, people from all over the world were cured of a psychiatric disease (Drescher, 2015). Differences in brain organization have been noted in terms of sexual orientation (Bao & Swaab, 2011; Byne et al., 2001), thus homosexuals and heterosexuals have different brain pattern in terms of hypothalamic activation. Would this be enough reason for categorizing homosexuality (or heterosexuality, for that matter) as a diagnosis?

On this topic, the latest edition of DSM-5 revealed an altered emphasis on the matter of gender identity. In some ways, this alteration has similarities with the historic decision made in 1973—when APA removed homosexuality as an illness from the DSM-5 (Drescher, 2015; Lev, 2013). The new emphasis is on the importance of distress rather than the before reiterated incongruity between body and mind. This alteration may render transsexual individuals from identifying with a pathological state if the gender identification does not cause the individual any distress. The newer emphasis in DSM-5 pretty much says that you can be transgender and psychologically healthy, or transgender and depressed. The sensation of gender dysphoria *per se* does not have to be the problem (although since DSM-5 claims comorbidity, this discussion would be something for future research). To close the loop, whilst comparing to when APA removed the diagnosis of homosexuality in 1973—the Nomenclature Committee agreed that homosexuality *per se* was not a generalized impairment (Drescher, 2015).

Since DSM-5 claims that comorbidity in gender dysphoria is common, a great question to answer would be to see if the distress which many experiences, might be

the result of a society and a culture which stigmatizes and discriminate (Fernández-Rouco, Fernández-Fuertes, Carcedo, Lázaro-Visa, & Gómez-Pérez, 2016; Smith et al., 2015), and or individuals who are not comfortable with the current gender roles (Coolidge et al., 2002)? What came first—the distress of the diagnosis or the judgmental treatment from sociocultural norms? A puzzling question, close to the chicken or the egg causality dilemma.

How then to handle this default situation with the cultural traditions and the designed societal gender roles? A remaining question would be how to act righteously is this argumentation of gender identity? The challenge would be to fully incorporate this knowledge and deconstruct heteronormative thinking. After all, we live in a society that is designed for the majority, but in 2017, one would hope that the discourse would go a little differently. Hence, that knowledge on variations in sex differentiation would be better known and available for the majority. Finally, some consider that no diagnoses will ever be enough to apprehend the great diversity of gender expressions and identities of mankind (Lev, 2013). However, with more aspiring knowledge through research and future findings—we ought to aim for a more accepting and well-prepared society in terms of gender identity.

The aim of this review paper was to present relevant findings and theories from the biological and neuroscientific community on the subject of gender identity and focus further on gender dysphoria. This paper tried, throughout provided research to answer the research question: *What are the neurobiological underpinnings of gender dysphoria?* Conclusively, there is biological evidence for sex differentiation and indications that lead science into considering biological components for gender dysphoria as well. As aforementioned, different degrees of normative and atypical brain development are habitual and part of the individual differences among natural

variation. Human beings possess different levels of estrogen and testosterone (Kandel et al., 1995). What we have concluded is how the level of feminization or masculinization of the genitals may not display the same level of the feminization or masculinization of the brain (Kandel et al., 1995; Swaab, 2007). The earlier described theory on why transsexuality develops result from the fact that sexual differentiation of the brain and differentiation of sexual organs develops during different time periods (Bao & Swaab, 2011; Berglund et al., 2008; Cohen-Kettenis & Gooren, 1999; Chung et al., 2002; Garcia-Falgueras & Swaab, 2008; Van Goozen et al., 2002; Kruijver et al., 2000; Luders et al., 2009; Smith et al., 2015; Swaab et al., 2001; Swaab, 2004; Swaab, 2007; Swaab & Hofman, 1995). Hence, the sexual organs appear well before the route of sexual differentiation of the brain in fetal development.

Additionally, hypothalamus has an important role in determining several sex differences, such as reproduction, sexual orientation and the identification of gender (Allen & Gorski, 1990; Byne et al., 2000; Swaab, 2007; Swaab et al., 2001; Swaab et al., 2003; Swaab & Hofman, 1988; Swaab & Hofman, 1995). Two important structures are attentive: data disclose how the INAH-3 volume and cell number could be dependent on gender identification since transsexual individuals had results that showed more similarities amongst the sex they claim they experience themselves, rather than their born, genetic sex (Garcia-Falgueras & Swaab, 2008). Furthermore, results showed how the BSTc in the MTF transsexual group was female-sized. The BSTc of the castrated men were in the typical male range (Swaab, 2004; Zhou et al., 1995). Moreover, MTF transsexuals had a larger volume of regional grey matter in the right putamen compared to the control men, which indicates a ”feminization” of the brain (Luders et al., 2009).

A discussion has been provided whether gender dysphoria should be considered a mental disorder at all. Looking at all the biological and neuroscientific findings it would make more sense for gender dysphoria to be a diagnosis of DSD—or perhaps, seen as a natural variation that doesn't have to be categorized as a disorder. Through historic validation, we have presented how gender dysphoria has existed longer than the contemporary diagnosis. *Two-spirits* and *fa'afafine* are great illustrations of variations in gender roles among other cultures, in time. The presented historical evidence shows us how transsexuality has existed long before the classification of mental illnesses. What this paper sought to provide was complementary ways of considering these variations as natural variations which nature has evolved, also to review if the classification of mental disorder is necessary and will serve a purpose in the future. Much has happened in terms of what to diagnose in less than a century, to learn from blunders is an absolute obligation in our informed society.

Byne et al. (2000) further claim how the understandings on brain development may be enhanced through studying differences among the sexual dimorphism spectra. Additionally, scientific interest among gender identity has increased over the years, especially since the 1990s (Bao & Swaab, 2011; Cohen-Kettenis & Gooren, 1999; Gooren, 2006; Smith, et al., 2015). From what has been settled from this paper, future research is recommended to focus more on the possible genetic factors of gender identity in order to bring upgraded facts to the table. Incomplete knowledge of what exactly constitutes an individual's gender identity is still actuality. Given the small sample sizes of studies on transsexual post-mortem brain material, the findings are hard to apply to the whole population; FTM transsexuality research especially needs larger sample sizes. Thus, more research is nowadays conducted on MTF transsexual.

The lack of statistical power affects the credibility of the studies. Furthermore, the research on transsexualism is mostly carried out on adults, more research on children is needed for clarifying how observed findings develop (early or later onset) (Smith et al., 2015). The results from Chung et al. (2002) needs further investigation and explanation. In future research, a multidimensional approach is desirable (Smith et al., 2015), regulating sexual orientation, age of onset, and psychiatric comorbidity. The connection between gender identity and genetics and/or interaction with sex hormones needs more research as well.

Moreover, due to differences in results between MTF and FTM transsexuals (Bentz et al., 2008), it is unsure if the female brain and the male brain show precisely the same brain activation patterns in terms of gender identity. Maybe, we ought to look for dissimilar arrangements, when investigating what is believed to be a gender identity network? Many inquiries are unanswered. In conclusion, opinions on the data on the transsexual brain as inhomogeneous indicate a need of more replications (Smith et al., 2015).

Given the issues at stake, this is something that needs urgent attention. Research has specified for us numerous examples of gender variations that display how one's gender identity is much less about choice and more about biological arrangements. Therefore, it can be concluded that an individual's sex, gender identity and gender expressions may vary. Scientific research has shown us that sex does not have to equal gender. The ongoing debate on what gender constitutes has to be modernized through recent findings, or as Darwin so nicely puts it (1871, p.4): "Ignorance more frequently begets confidence than does knowledge: it is those who know little, and not those who know much, who so positively assert that this or that problem will never be solved by science".

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Appendix

Gender dysphoria in children, 302.6

A. ”A marked incongruence between one’s experienced/expressed gender and assigned gender, of at least 6 months’ duration, as manifested by at least six of the following (one of which must be Criterion A1):

1. A strong desire to be of the other gender or an insistence that one is the other gender (or some alternative gender different from one’s assigned gender).
2. In boys (assigned gender), a strong preference for cross-dressing or simulating female attire; or in girls (assigned gender), a strong preference for wearing only typical masculine clothing and a strong resistance to the wearing of typical feminine clothing.
3. A strong preference for cross-gender roles in make-believe play or fantasy play.
4. A strong preference for the toys, games or activities stereotypically used or engaged in by the other gender.
5. A strong preference for playmates of the other gender.
6. In boys (assigned gender), a strong rejection of typically masculine toys, games and activities and a strong avoidance of rough-and-tumble play; or in girls (assigned gender), a strong rejection of typically feminine toys, games and activities.
7. A strong dislike of one’s sexual anatomy.
8. A strong desire for the primary and/or secondary sex characteristics that match one’s experienced gender.

B. The condition is associated with clinically significant distress or impairment in social school, or other important areas to functioning.

Gender dysphoria in adolescents and adults, 302.85

A. ”A marked incongruence between one’s experienced/expressed gender and assigned gender, of at least 6 months’ duration, as manifested by at lease two of the following:

1. A marked incongruence between one’s experienced/expressed gender and primary and/or secondary sex characteristics (or in young adolescents, the anticipated secondary sex characteristics).
2. A strong desire to be rid of one’s primary and/or secondary sex characteristics because of a marked incongruence with one’s experienced/expressed gender (or in young adolescents, a desire to prevent the development of the anticipated secondary sex characteristics).
3. A strong desire for the primary and/or secondary sex characteristics of the other gender.
4. A strong desire to be of the other gender (or some alternative gender different from one’s assigned gender).
5. A strong desire to be treated as the other gender (or some alternative gender different from one’s assigned gender).
6. A strong conviction that one has the typical feelings and reactions of the other gender (or some alternative gender different from one’s assigned gender).

B. The condition is associated with clinically significant distress or impairment in social, occupational, or other important areas of functioning.