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Abstract

Background: Biological causes underlying gender incongruence are still largely unknown and have received increased attention in the last years. Several brain structures in transgender people resemble those of the gender which they identify more closely with compared to those of their own chromosomal sex. An important role in several brain processes, especially for the regulation of emotion processing, plays the serotonergic neurotransmitter system. The serotonin transporter (SERT) modulates multiple brain functions and is involved in several psychiatric disorders. Here, we examined genetic effects of the SERT short/long allele on binding potential (BP_{ND}), an index of protein density, in cis- and transgender subjects. In addition, we investigated epigenetic pattern and single nucleotide polymorphisms (SNPs) in the SERT gene and serotonin receptor 2A gene (HTR2A) as well as effects of methylation on major/minor alleles between women and men.

Methods: Twelve female-to-male (FtM) and 14 male-to-female (MtF) transsexuals (persons with gender dysphoria), as well as 11 female (FC) and 15 male (MC) controls were included in this study. Subjects underwent a [^{11}C]DASB positron emission tomography (PET) scan and were genotyped using a MassARRAY MALDI-TOF platform and the Sequenom iPLEX assay to find effects of SERT short/long allele on BP_{ND} between transsexuals and healthy controls. For the analysis of SERT DNA methylation levels, a total number of 90 subjects were included, 38 males and 52 females. Differences between the sexes in SERT methylation levels and effects of major/minor alleles on methylation patterns were examined as well as associations between methylation levels and *in vivo* SERT expression. SPSS was used for statistical analyses.

Results: Effects of SERT genotype showed a higher SERT BP_{ND} in the long allele carriers for the MtF group compared to other groups. FtM short allele carriers had lower BP_{ND} compared to the MC group and a higher BP_{ND} compared to FC. Genotype effects of the SNP rs6311 on methylation levels were detected in females for several CpG sites with higher methylation levels in the major allele. In males in contrast, genotype effect was observed for minor allele carriers as having higher methylation compared to major allele carriers.

Conclusion: The present findings support the combination of neuroimaging techniques and genetic methods for a better understanding of sex differences. However, for further investigations including larger sample sizes is suggested in order to identify sex differences in genetic and epigenetic patterns.

Zusammenfassung

Hintergrund: Die biologischen Ursachen der Gender Inkongruenz sind noch weitgehend unbekannt und haben in den letzten Jahren zunehmend an Interesse gewonnen. Mehrere Gehirnstrukturen bei Transsexuellen ähneln stärker dem Geschlecht, mit dem sie sich näher identifizieren, als ihrem chromosomalen Geschlecht. Eine wichtige Rolle bei verschiedenen Gehirnprozessen, insbesondere bei der Regulierung der Emotionsverarbeitung, spielt das serotonerge Neurotransmittersystem. Der Serotonin-Transporter (SERT) moduliert mehrere Gehirnfunktionen und ist an einigen Krankheiten beteiligt. Hier untersuchten wir die genetischen Auswirkungen eines Längenpolymorphismus des SERT Gens auf das Bindungspotenzial (BP_{ND}), einem Index für die Dichte eines Proteins, bei weiblichen und männlichen Cis- und Transsexuellen. Weiters untersuchten wir vergleichend epigenetische Muster und Einzel-Nukleotid- Polymorphismen (SNPs) im SERT-Gen und Serotonin-Rezeptor-2A-Gen (HTR2A) sowie Major/Minor-Allel bei Frauen und Männern.

Methoden: Zwölf Frau-zu-Mann (FtM) und 14 Mann-zu-Frau (MtF) Transsexuelle (Personen mit Geschlechtsdysphorie) sowie 11 weibliche (FC) und 15 männliche (MC) Kontrollpersonen wurden in diese Studie einbezogen. Die Probanden wurden einem [^{11}C]DASB Positronen-Emissions-Tomographie (PET) Scan unterzogen und ihr Genom auf einer MassARRAY MALDI-TOF-Plattform mit dem Sequenom iPLEX-Assay genotypisiert, um die Auswirkungen des Längenpolymorphismus des SERT–Gens auf BP_{ND} zwischen Transsexuellen und gesunden Kontrollen zu finden. Für die Analyse der DNA Methylierung wurden insgesamt 90 Probanden, davon 38 Männer und 52 Frauen, eingeschlossen. Unterschiede zwischen den Geschlechtern in der SERT Methylierung und die Effekte der Methylierung auf Major/Minor-Allele wurden ebenso untersucht wie Zusammenhänge zwischen der Methylierung und der in vivo SERT Expression. SPSS wurde für die statistische Analysen eingesetzt.

Ergebnisse: Effekte des SERT Genotyps zeigten ein höheres SERT BP_{ND} in dem Lang-Allelträgern für die MtF-Gruppe im Vergleich zu anderen Gruppen. FtM Kurz-Allelträger wiesen ein niedrigeres BP_{ND} im Vergleich zur MC-Gruppe und ein höheres BP_{ND} im Vergleich zu FC auf. Effekte von SNP rs6311 auf den Methylierungsgrad wurden bei Frauen für mehrere CpG-Stellen mit höheren Methylierungswerten im Hauptallel nachgewiesen. Bei Männern hingegen wurde ein Effekt beim Minor-Allel mit einer höheren Methylierung im Vergleich zu Major-Allelträgern beobachtet.

Schlussfolgerung: Die vorliegenden Ergebnisse unterstützen die Kombination von Neuroimaging-Techniken und genetischen Methoden zum besseren Verständnis von Geschlechtsunterschieden. Für weitere Untersuchungen wird jedoch eine größere Stichprobe vorgeschlagen, um Geschlechtsunterschiede in genetischen und epigenetischen Mustern zu identifizieren.

1. Background

1.1 Female – male

Men Are From Mars, Women Are From Venus! (Gray, 1992). The symbolic metaphor from John Gray describes the difference between male and female in an amusing way like (almost) nothing else. Everyone is aware that there exists a wide range between men and women, which start by biological and anatomical distinctions to fundamental psychological differences in social behavior. The chromosomal sex is already known at the time of fertilization. In the egg cell the sex chromosome contains X, the sperm X or Y. Egg and sperm merge and new life forms typically with XY (boy) or XX (girl) combination. Charles Darwin's theory describes already the sexual selection of men and women by pursuing survival through reproduction (Darwin, 1871). Some important advances, like the characteristic of sexual features and clarification of sex and gender, have been achieved since Darwin's work (Trivers, 1972). Several basic differences are visible at first glance. The primary sexual characteristic is obvious and already developed at birth, such as the external genitalia. The secondary sexual features are formed during puberty by the action of hormones (Schonfeld, 1943). Gender-specific behaviors and feelings are counted among tertiary sexual characteristics (Ellis, 1934).

1.1.1 Gender Roles

People are born female or male, who differ by chromosomal sex, but grow into gender roles, behavior and identity (Geary, 1998). Children are embossed from their parents and social environment and learn typical stereotypes and roles. So far, there are no known cultures without gender roles (Costa et al. 2001). While in the 18th century the term gender character was used (Hausen 2014), during the 20th century the concept of gender role has emerged more and more. Therefore, femaleness incarnates domestic life, devotion, appreciation and empathy. On the other hand, masculinity stands for strength, powerfulness, assertiveness and authority. Most of the differences that do exist are the result of gender roles, not the cause (Basow 1992). The social construction of gender is described as gender differences in societies and roles are prescribed as ideal or appropriate behavior for a person of that specific sex (Lindsey, 2015).

1.1.2 Sex differences of the human brain

Differences between males and females are not only found in body anatomy and hormone composition, but the brain also shows sexual dimorphism. Sex-related differences are already recognized during the early development of the nervous system in embryos (Xu et al. 1992). The progression of embryonic structure into the future central nervous system is called neurulation (Schoenwolf & Smith 1990). It is a developmental mechanism in which the ectoderm above the chorda thickens into neural beads and forms the neural tube after sinking into a neural groove (Benninghof 1985). Consequently the brain and spinal cord have evolved and continue with the formation of cortical structures. It is characterized by a layer-like arrangement of nerve cells. Corticalization primarily represents an enlargement of the brain surface by increasing the total number of nerve cells and is also an expression of the differentiation level of the cortical parts of the telencephalon (Corticalisation - Lexikon der Neurowissenschaft 2018). The brain is the slowest-growing organ in the fetal mammal (Sacher & Staffeldt 1974), however the brain growth occurs in the same order in the two sexes (Miller & Corsellis 1977). Sex differentiation of the brain develops in the second half of pregnancy (Swaab & Garcia-Falgueras 2009).

The surface of the brain anatomy was already described in the 19th and 20th century by pioneers like Eberstaler (1884), Cunningham (1892), Déjerine (1895), Zuckerkandl (1903) or Elliot-Smith (1907). They already portrayed sexual dimorphism in shape and surface area of the brain. Fundamentally the brain weighs more in males than in females by 9.8% (Dekaban & Sadowsky 1978). Research has indicated that men have a larger brain volume compared to their female counterparts (Luders & Toga 2010), but women's cortical thickness is greater than in men (Luders et al. 2006). There are exceptions, such as the female splenium is bigger and more bulbous than in men (DeLacoste-Utamsing & Holloway 1982). A voxel-based morphometry study demonstrated that human males showed larger gray matter volumes in left amygdala, whereas females had larger right striatal and bilateral hippocampal gray matter volumes (Neufang et al. 2009).

However, the differences are not only noticeable in size and shape of the whole brain but also in behavior. The sexually dimorphic nucleus of the preoptic area, which is involved in male copulatory behavior (Morris et al. 2004), is 2.6 times larger in males (Gorski et al. 1980). In contrast, females have a larger anterior-ventral periventricular nucleus with higher cell density (Bleier et al. 1982) that regulates

luteinizing hormone level responsible for copulatory behavior in females (Tsukahara 2009) and males (Rhees et al. 1999). Moreover, sex differences are also present by the amount of synapses in the ventromedial hypothalamic nucleus. Scientists have suggested that females have less synapses compared to males in this area (Matsumoto & Arai 2008), which control lordosis, mounting and norepinephrine release (Etgen & Morales 2002). In addition, females have 20% fewer dopaminergic neurons (Dewing et al. 2006), which are an important part of the motor activity control system (Groenewegen 2003).

Beside the observed differences in brain morphology, the connectivity has been studied extensively over the last decades as well. In relation to functional connectivity women show a higher **inter**hemispheric connectivity whereas men brains have a higher **intra**hemispheric connectivity (Ingalhalikar et al. 2014; Szalkai et al. 2015). According to this, female brains have an increased connection between the two halves of the cerebrum and males have an increased connection within each brain hemisphere,

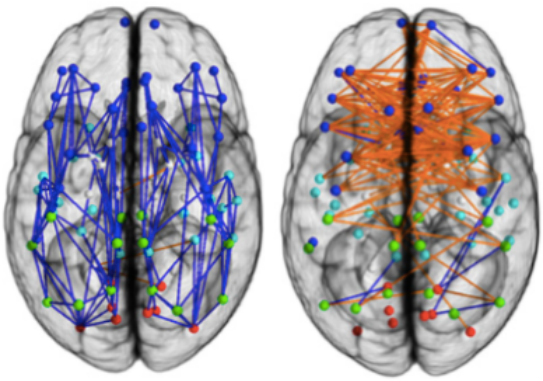

Figure 1: Brain connections. Male brain is depicted on the left side, showing increased intra-hemispheric connections (blue). Female brain is depicted on the right brain and shows higher inter-hemispheric connections (orange). Photograph issued by National Academy of Science. Taken and adapted from (Ingalhalikar et al. 2014)

Levy & Heller described sex differences in hemispheres and accented genetic and hormonal effects in human neuropsychological pattern. The anterior and posterior cortical organization such as cognitive abilities are different in females and males (Levy & Heller 1992). Each area is associated with a particular function, for example the left cerebral cortex, especially frontal, temporal and parietal lobes, are

linked to languages (Binder et al. 1997), the visual cortex to spatial attention (Gandhi et al. 1999) and the parietal cortex to hand, arm and head movements (Rizzolatti et al. 1997). Therefore, it is not surprising that men and women have different strengths and distinct predispositions based on various information processing. Scientists have consistently found more and more evidence for sex-related differences in the brain and in their resulting functions. For example, speaking patterns are partly different in males and females. Most women try to assemble connections and negotiate relationships during conversations, while men establish and maintain status in a hierarchical social order (Tannen 1991). Furthermore, studies indicate that female subjects have higher performance in emotion processing tasks compared to males (Grossman & Wood 1993; Kring & Gordon 1998) and similarly men and women show significant differences when processing cognitive tasks. While male participants excel in quantitative and visuospatial abilities by comparison to female performance, verbal skills were better pointed out by women than men (Halpern et al. 2007).

The brain anatomy and outcome of mental activity and thinking are diverse, even if men and women are more alike than they are different. In 2005, the psychologist Janet Hyde published a meta-analysis of gender differences in thinking, communication style and personality. This overview of various studies advanced the gender similarity hypothesis. Boys and girls, as well as men and women are similar on most, but not all psychological variables (Hyde 2005).

There are clearly demonstrable differences in the cognitive field, for example, when it comes to spatial perception, but there should be no distinguishable difference in the majority of cognitive performance and general intelligence (Hirnstein et al. 2014). Generally, men and women differ in mean cognitive values, however the overlap area between the groups is very large. The range of benefits within the sexes is much wider than the average difference. Thus, the two sexes have similar brains with multiple sex-specific differences.

1.2 Transgender

Most children learn to identify themselves by their chromosomal sex at the age of three (Pate, 2012). However, to be a woman or a man is the result of group processes, social environment and culture. The biologist Anne Fausto-Sterling had shown that genes, chromosomes and hormones alone are not enough to program male or female development (Fausto-Sterling 1993). The distinction between sex and gender has become widespread. On the one hand, sex refers to the biological gender, which is determined by external sex characteristics. Gender is usually referred to the social role, the stereotypical behavior of women and men. Nevertheless, ‘man’ and ‘woman’ doesn’t imply absoluteness, a person who always act clichéd and have a preference for the opposite sex. Therefore, concepts of cis- and transgender were introduced (Sigusch 2005). Cisgender people are individuals whose gender identity matches the gender assigned at birth and “the male or female sex are typical of the social category of man or woman” (Aultman 2014).

Figure 2: Transgender Symbol.
A combination of Venus (♀ - female) and Mars (♂ - male) symbol with additional "arm" for transgender.

Person’s self-perceived feeling of gender does not always match the biological sex. If this sensation is accompanied by psychological stress, uncomfortable feelings with their body and/or lifetime-associated disorder, it is called gender dysphoria (American Psychiatric Association 2013). Over the last years, the expression “dysphoria” was slowly changed by the term gender “incongruence”. Estimates for the prevalence greatly vary as demonstrated in a considerably large study from 2009 where the prevalence rate was 4.26 out of 100.000 people in Germany (Meyer zu Hoberge 2009). Nowadays the prevalence is specified to be 5.5 out of 100.000 individuals in Austria as reported by the Bioethikkommission of Vienna in 2017. Up to 0.8% of the population consider themselves as gender incongruent and contemplate a sex change surgery (Kuyper & Wijsen 2014). Recently, the World Health Organization changed the categorization of transgender people. Since July 2018 they should no longer be considered as mentally ill. The term gender incongruence is now listed under “conditions related to sexual health” (Winter et al. 2016).

However, the causes for gender incongruence are still largely unknown and speculative (van Trotsenburg 2018). The literature of possible mechanisms and basics are extremely limited. Therefore, a few twin case studies were executed, with controversial concordances rates (Segal 2006; Hyde & Kenna 1977; Sadeghi & Fakhrai 2000). A closer view of genetic basis could help at this point. Environmental influences of genetics can enable a clear support for transsexualism. Studies have investigated chromosomal abnormalities in people with gender incongruence and found a high frequency (for example with 47,XXX or aneuploidy), but no statistically significant association (Snaith et al. 1991; Haberman et al. 1975; Buhrich et al. 1978). Furthermore, in recent years some possible genes and polymorphisms for transsexualism were examined (Bentz et al. 2008; Henningsson et al. 2005), these will be described in detail later on. Overall, the significance of these studies is vague, findings are controversial and due to the fact of very small sample sizes, the findings remain unclear (Ngun et al. 2011).

1.2.1 Brain differences

It is suggested that hormones play an important role during prenatal development, leading to an independent differentiation of brain and primary sex characteristics (Bao & Swaab 2011). Recent neuroimaging studies have evaluated sex dissociations in the brain of people with gender incongruence. The results suggested that some structures and mechanisms in transgender brains resemble people who identify more closely to people of their experienced gender than people of their chromosomal sex (Guillamon et al. 2016). Morphometry studies have found that the male-to-female (MtF) group shows increased interhemispheric lobar connectivity weights (LCWs) and decreased intrahemispheric LCWs in female-to-male (FtM) transsexuals (Hahn et al. 2015). In comparison to the results from Ingalhlikar (Ingalhalikar et al. 2014), it seems that MtF have similar interhemispheric connectivity to males. MtF have increased cortical thickness (CTh) in sensorimotor areas in the left hemisphere as well as in the right orbital, temporal and parietal areas (Luders et al. 2012) that are similar to women. Zhou and colleagues were the first who specified sex disparities in the central subdivision bed nucleus of stria terminalis in humans as a potential biological marker for gender identity (Zhou et al. 1995). A recently published study

showed specific brain connectivity for people with gender incongruence. Untreated FtM show a different functional connectivity in own-body perception and self-referential processing compared to male and female controls (Manzouri et al. 2017). In contrast to males, the CTh in FtM was found to be thicker in the temporal and parietal regions - similar to control females - and they showed a larger volume of putamen that did not differ from males (Zubiaurre-Elorza et al. 2013). Furthermore, there exists a difference in mean diffusivity in almost all white matter tracts between transsexual people and controls. Female controls had the highest values followed by FtM, then MtF and male controls exhibiting the lowest scores (Kranz et al. 2014). It seems that those network parameters may represent unique characteristics of gender identity persons, whereas local physiological aspects reflect the actual gender identity.

Therefore, a few studies have concluded that people with gender incongruence have similar sensory perception and the aesthesia is converting to the gender which they identify more closely with. For visual sex cues MtF and control women show the same brain activation (Gizewski et al. 2009). It was suggested that transsexual people have an increased functional connectivity between the ventral tegmental and anterior cingulate cortex after erotic stimuli, a pattern which cannot be seen in male or female controls (Ku et al. 2013). The connectivity is marked by a fast information transfer through hormones. In body processes, there may exist a difference between the regulation in the nervous system and the biochemical way in the hormonal system.

1.3 Hormones (Molecular communication)

The exchange of information within the body is characterized through a slower biochemical regulation by hormones. A hormone is defined as an “organic substance, which influences physiological processes and body functions like growth, differentiation and development” (Davies 2010). Hormones, which belong to the important class of messenger molecules that are produced in highly specialized endocrine glands, are distributed in the body by the circulatory system via diffusion and the bloodstream. Many hormones are already very effective in very low concentrations and regulate important body processes as well as the formation of sexes (Kleine & Rossmanith 2016).

Hormones can be divided into three major classes. Firstly, the endocrine system, which controls the organs by secreting hormones from peripheral endocrine glands through the bloodstream (Löffler 2008). After delivery, these hormones act in a completely different body part. The endocrine system is very closely linked to the second class, the neuroendocrine system, in which neuronal information transmission is conducted by hormones. The close connection becomes obvious through the fact that nerve cells can produce hormones, and transmitters activate hormone forming cells (Zilles & Rehkämper 1993). Finally, there is the autocrine paracrine system (cell-to-cell communication), in which hormones influence neighboring cells or even itself by feedback mechanisms and take effect locally. Neurotransmitters have also a paracrine effect, because they are produced by nerve cells and released into the synaptic cleft. Generally, it is believed that the hormone system is not directly regulated, however it is dependent on receptor selectivity. The mechanism of “the lock and key” model is important for the accuracy as well as the enhancement of effector molecules. All hormones that are supposed to keep a certain blood level of a substance constant, are not influenced by the central nervous system (CNS), but by the concentration of the substance itself (Horn 2009). Some glands like the thyroid, pancreas, adrenal glands or gonads produce crucial hormones that impact body processes and organs in the same way the whole life. Others substances have to change their concentration during the development of the organism like sex hormones. Therefore, they underlie the regulation of the CNS via the hypothalamus pituitary system. The hypothalamus acts as an integrative center that receives a wealth of control signals from almost all areas of the sensory and autonomous system (Offermanns 2012). The processing of the hypothalamus leads

to hypothalamic control signals in the form of the release of neurotransmitters and hormones, which mainly affect the pituitary gland (Fig 3). For example, the gonadotropin-releasing hormone (GnRH) is a hormone in mammalians, which were produced in the hypothalamus and stimulate the pituitary gland. These in turn distribute luteinising hormone (LH) and follicle-stimulating hormone (FSH) that regulates the function of ovaries and testis. This is a feedforward-mechanism - with stimulation and inhibition - which hardly change in men, but vary vigorously in women (Ehlert 2011).

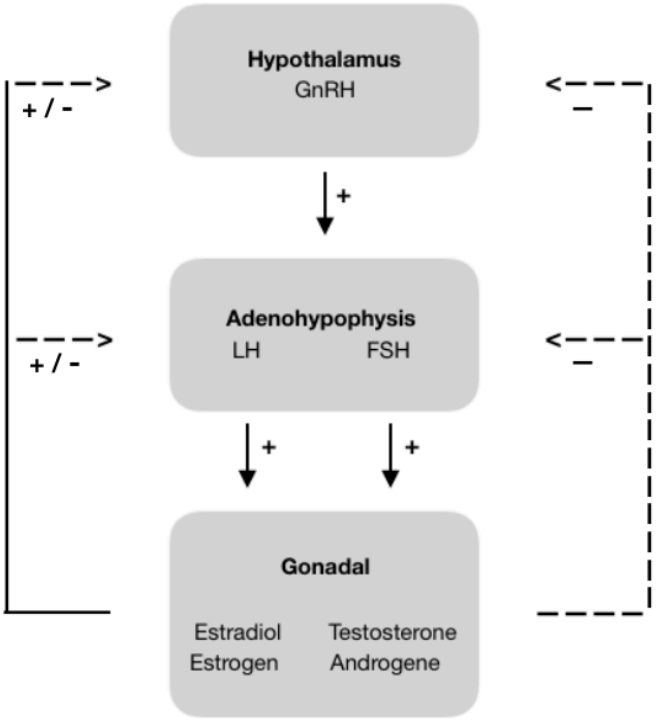

Figure 3: The control mechanism of the reproductive axis between hypothalamus, pituitary gland and gonadal.
GnRH Gonadotropin-releasing hormone; *LH* luteinising hormone; *FSH* follicle-stimulating hormone.
→ stimulation; - -> inhibition

Hormones, can be differentiated by their chemical structure. The three types are amino acid derivatives, peptides and steroid hormones. Eicosanoids and glycoproteins are an exception, which play also an important role in the body (Horn 2009). In the following section, sex hormones, which are a subgroup of steroid hormones will be discussed in more detail.

1.3.1 Sex hormones

The lipophilic steroid hormones are of particular importance for the topic of differentiation in the sexes. All hormones, male or female, can be detected in both men and women (see Fig 3). Their concentration in the blood and the ratio of hormones to each other is determining the sexual specificity (Horn 2009). A large part of steroids is transported into the blood stream with carrier proteins, the so called sex hormone binding proteins (SHBG) and corticosteroid binding globulin (CBG) (Siiteri 1979). At their destination, they bind to their corresponding intracellular receptors that leads to a change in the configuration and unfold effects. Gender specific hormones are androgens, typical for male characteristics, while gestagen and estrogens have preponderant feminising effects (Selye 1941). Androgens like testosterone are required for development of male sex characteristics and promote the growth of muscles and body hairiness (MacLean et al. 1993). Estrogen and gestagen are crucial for the reproductive function and menstrual cycle of women. After puberty, the regulation of the distribution of sex hormones in women is more complex compared to men. The hormone level is depended on the menstrual cycle (Reed & Carr 2015).

1.3.2 Neurotransmitter (Electrical communication)

As mentioned before, molecular communication is common in biological systems and the endocrine and neuroendocrine systems have some similarities and overlap (Bleich et al. 1982). That is why, neurotransmitters similar to hormones are carriers, which act in the brain only between two nerve cells. Neurotransmitters are a type of chemical messenger which belong to the nervous system that is stored in vesicles in nerve endings. After release at the membrane at the presynaptic side it diffuses across the synaptic cleft (the small gap between the synapses of neurons) to the postsynaptic membrane. They act very fast, usually within a few milliseconds (Johnson & Koerner 1988).

Subsequently each carrier molecule binds to a different receptor on the effector cell. Depending on the receptor, a messenger substance can inhibit or exhibit nerve cells through an electrical signal, called an action potential (Lodish et al. 2000). The target cells then open or close various ion channels, which sets further reactions in

motion. An excitatory transmitter promotes an action potential in the receiving neuron, through increasing conductibility for cations (Na^+/K^+), resulting in depolarization. On the other hand, an inhibitory transmitter prevents it through increasing the conductibility of K^+ and CL^- that causes hyperpolarization (Bear et al. 2007). The effect of neurotransmitters is limited only briefly and locally, as they are immediately degraded and/or absorbed again (Purves et al. 2004). Some neurotransmitters cannot be assigned exclusively to one group and are therefore referred as completely effective neurotransmitters like noradrenalin, dopamine or serotonin (Horn 2009). For the relevance and scope of this project, serotonin will primarily be discussed in the following sections.

1.3.3 Serotonergic system

Serotonin (5-hydroytryptamine, 5-HT, SER) is a hormone and a neurotransmitter, which is found in plant, fungi, coelenterates, mollusks, arthropods, vertebrates and in edible fruits and nuts (Tyce 1990). About 95% of the serotonin is arranged in the gastrointestinal tract and the remainder is found in the brain (Kim & Camilleri 2000). Particularly high concentrations within the CNS are synthesized in the midbrain raphe nuclei and released in terminal areas of the forebrain (Adell et al. 2002). Serotonergic raphe neurons project largely throughout the CNS and to nearly all areas (Fig4). For instance, neurons of 5-HT innervate the hypothalamus, basal ganglia and limbic structures.

Serotonin is synthesized from the amino acid tryptophan by two enzymatic steps. First, the hydroxylation of tryptophan produces 5-hydroxytryptophan and in the second step, serotonin will be produced through decarboxylation (Tyce 1990). Seven main types of serotonin ($5\text{-}HT_x$) receptors have been identified by a combination of pharmacological techniques and molecular cloning (Barnes & Sharp 1999) and there are about 14 different receptors highly selective for serotonin. For example, the $5\text{-}HT_{1A}$ receptor in the midbrain raphe region regulates signaling by modulating serotonergic cell firing (Sharp & Hjorth 1990). In contrast, signaling of $5\text{-}HT_{1A}$ receptor on glutamatergic and GABAergic neurons has inhibitory effects on non-serotonergic neurons (Hahn et al. 2010).

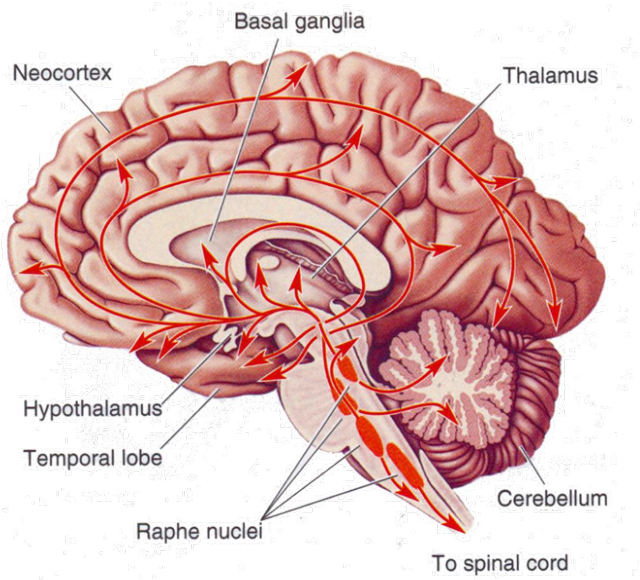

Figure 4: The serotonergic System. Serotonergic raphe neurons project to virtually all parts of the brain, such as temporal lobe, hypothalamus, neocortex and cerebellum. Taken and adapted from (Bear et al. 2007).

All 5-HT receptors are coupled to G-proteins, with the exception of 5-HT_3 receptors, which acts as a cation-permeable ion channel (Millan et al. 2008). The inactivation of serotonin happens through the resumption over Na^+-dependent amine transporters, followed by repeated storage in vesicles or metabolized by monoamine oxidase A (MAO-A) (Heath & Hen 1995). The decomposition products will be segregated by urine (Schön et al. 1960).

Early body development is already affected by serotonin through its function as a growth factor (Whitaker-Azmitia et al. 1995), as it regulates cell division, outgrowth, differentiation, synaptogenesis and dendritic pruning (Gaspar et al. 2003). Serotonin regulates the development on its own and function as a growth factor (Bonnin et al. 2007; Brummelte et al. 2017). Furthermore, serotonin acts as a peripheral neurotransmitter, which modulates body processes like thermoregulation (Gudelsky et al. 1986) and vasoconstriction (Yildiz et al. 1998). In the adult, serotonin influences various brain functions like emotion (Lesch 2007), cognition (Canli & Lesch 2007) and motor function (Bharucha et al. 2000) as well as neuroendocrine

functions like food intake (Meguid et al. 2000), sleep (Ursin 2002), circadian rhythms (Martinowich & Lu 2008) and steroid hormones such as cortisol and progesterone (Lanzenberger et al. 2010). However, 5-HT functions cannot be strictly separated between early development and later postnatal, childhood or adult time frames, because the transitions are flowing (Brummelte et al. 2017).

Over the last decades some studies have illustrated the influential role of 5-HT in health and illness. 5-HT was firstly linked to depression (Langer et al. 1981), shortly after its identification as a target of antidepressant drug (Raisman et al. 1979). It seems that serotonin plays a key component in a huge number of neuropsychiatric disorders, like major depression, schizophrenia, anxiety, autism and affective disorders (Spies et al. 2015; Lanzenberger et al. 2007; Whitaker-Azmitia 2001; Sodhi & Sanders-Bush 2003; Bonnin & Levitt 2012).

1.3.4 Neurochemical and behavioral sex differences

Generally, a lot of scientific evidence highlights the hormonal differences in females and males. From early on, the brain development determines sex differences at a much later stage than the establishment of genitals (Bao & Swaab 2011). Indications for sex dimorphic and temperamental sex differences are predestinated by hormones (Ehrhardt & Meyer-Bahlburg 1981). Sex specific behavior are triggered by diverse hormone levels during the pregnancy (Hines 1982). The organizational effect, which is responsible for the development of brain structures, mostly happen in the prenatal phase, whereas activational effects are induced by changes in hormone concentration over the span of lifetime and can be reversed (Phoenix et al. 1959; Swaab 2007). Sex steroid hormones such as testosterone and estrogen seem to play an important role in the organization and activation of brain areas. Experiments in male rodents showed the direct influence of sex hormones on the developing fetal brain. Androgen treated rats will develop a masculine sex behavior pattern following the dispense of testosterone in adulthood (Döhler 1991). In addition, the postnatal hormonal level influences the sex-specific function and behavior. Female rats were treated with sex hormones during the sensitive period in the first weeks after birth and a changing steroid concentrations in brain regions, unrelated to circulating steroids, was shown (Konkle & McCarthy 2011).

Moreover, studies in humans have shown the influence of sex hormones on some diverse perceptions of men and women. It was implied that differences in steroid hormones modulate the sensitivity of pain and analgesia (Pickering et al. 2003; Craft et al. 2004). In women it was shown that a high testosterone level leads to increase in verbal aggression and impulsivity (Cashdan 2003; Von Pahlen et al. 2002).

Testosterone is the major factor for development of male gender identity and heterosexual orientation (Swaab 2004). The density of steroid hormones in varied brain regions likely plays a crucial role for structural differences. It could be observed in a meta-analysis that the amygdala of men are larger and exhibit a higher density of androgen compared to estrogen receptors, while women's hippocampus are larger with an inverse density ratio (Ruigrok et al. 2014). A recent study showed that hormonal variations correlate with altered white matter structure. Transgender people represent an androgenization-related reduction (FtM) or rather a feminization-related increase (MtF) in mean diffusivity after hormone replacement therapy (Kranz et al. 2017). This finding indicate altered brain structures based on sex hormones.

Regarding gender identity and sexual orientation, hormones are crucial for brain development and alterations might provide possible biological explanations. The sex steroid hormones are influenced by a multitude of factors which also affects the serotonergic system (Barth et al. 2015). Several animal and human studies showed sex specific differences in multiple levels in the serotonergic system (Nishizawa et al. 1997; Weiss et al. 2005; Staley et al. 2001; Mitsushima et al. 2006). It has been pointed out that levels of indolamine synthesis are consistent in the brain, but serotonin concentrations changes in different brain areas. Nishizawa and colleagues found a 52% higher rate of synthesis in males than in females (Nishizawa et al. 1997).

Recent animal models indicate that the 5-HT transporter (5-HTT) is necessary for male sex preference (Liu et al. 2011). This issue is though controversial and other groups have shown seemingly opposite findings: male mice lacking serotonin in their brains still prefer female mice (Brookshire 2015). It is still unknown whether removing serotonin from the brain leads to a loss of sex preference. It is controversial discussed that the role of serotonin influences just the social and not sex behavior. Testosterone and estrogen treatment in humans have a strong influence on serotonin transporter binding which has been studied in transgender people. Studies of serotonin transporter binding demonstrated that the binding potential in FtM

transsexuals and control females are similar, but clearly differ from men (Kranz et al. 2013). Antiandrogen and estrogen treatment in MtF transsexual subjects lead to a decrease in 5-HTT binding in the insula and the anterior- and mid-cingulate cortices (Kranz et al. 2015). Additionally, the increase of testosterone level in plasma is significantly correlated with an increase in 5-HTT binding in FtM.

In which way sex hormones are modulating serotonergic neurotransmission and resulting in changes in serotonergic receptor and transporter expression via genomic mechanisms remains unclear and questionable. Understanding the pathway is an important route to decode the physiological discrepancy between females and males.

1.4 Epigenetics

Besides the differences in brain development, morphology and connectivity, it has been frequently reported that genetic variants of proteins involved in serotonergic transmission influence the serotonergic system in a distinct way. A response to environmental influences, indicated by a link between nature (genetic) and nurture (life experience), is reflected through epigenetic modification (Schneider et al. 2017). This mechanism can cause alterations to the gene function and activity without changing deoxyribonucleic acid (DNA) sequence (Moore et al. 2013). Epigenetics describes the meta-level of genetic regulation beyond the stored information in DNA. Those are heritable reversible mitotic and/or meiotic transformations (Schuebel et al. 2016), which change the final outcome of locus or chromosomal regions. There are many pathways, such as “DNA methylation, nucleosome or chromatin remodeling, histone modifications, exchange of histone variants and non-coding RNAs that together contribute to differences of the chromatin template” (Jenuwein 2006). A comparison of genetic and epigenetic differences is illustrated in figure 5. Chromatin condensation and opening as well as shortening and lengthening of telomeres are reversible and thus offer a rapid adaption to environmental changes (Schuebel et al. 2016). DNA methylation changes, as it is one of the most important epigenetic modification (Jeltsch 2002), willbe explaining in detail in the following section

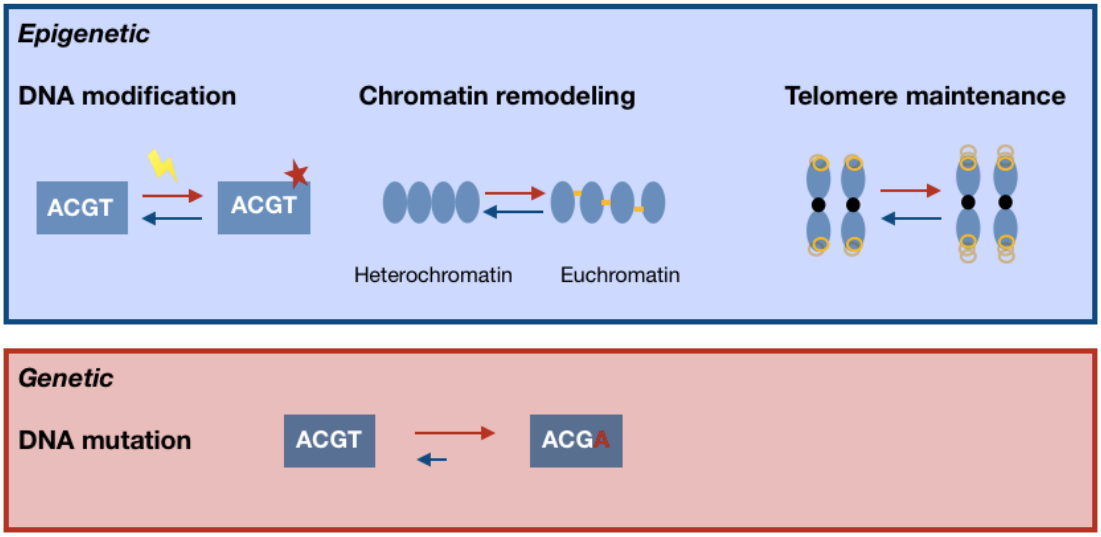

Figure 5: Epigenetic and genetic alterations of DNA. Exemplary DNA modification, chromatin remodeling and telomere maintenance are itemized for epigenetic. DNA mutation are exemplified for genetic variation. Taken and adapted from (Schuebel et al. 2016).

1.4.1 Methylation Pattern

Epigenetic processes like DNA methylation mark the interaction between genes and the environment. During methylation a methyl group is added to the cytosine's fifth carbon ring (Fig 6), which usually occurs at cytosine-phosphate-guanine sites (CpG) resulting in 5-methylcytosine (Bock 2012). It is a heterocyclic organic compound with a pyrimidine backbone. 5-methylcytosine is a derivative of the nucleic base cytosine with an additional methyl group in position 5.

Figure 6: Methylation. Cytosine is added to a methyl group at the 5-cytosine ring, thus resulting in 5-methylcytosine.

Epigenetic modifications affect the course of exposure to gonadal hormones organized in the brain that induces a sexual dimorphic response (McCarthy et al. 2009). This change can be provided by certain mechanisms, which have an impact on the spectra of human personalities as well as their behavior, susceptibility to health risks or stress induced response. In animal studies it was found that resilient mice develop an antidepressant-like epigenetic mechanism to chronic stress (Wilkinson et al. 2009). Therefore, the connection between methylation of 5-HTT and emotion response is highly interesting. Especially various methylation levels of the 5-HTT promoter (5-HTTLPR) are linked with sensation and emotion processing.

Therefore, it isn't surprising that some studies on the 5-HTT promoter have demonstrated the association to other factors such as acute stress (Kang et al. 2013), history of lifetime depression (Philibert et al. 2008), depression severity (Okada et al. 2014) and antidepressant response (James et al. 2017). In the last decades, researchers have found more and more epigenetic dysregulations linked with diseases. Some experiments found a link between psychiatric disorders, amygdala reactivity and methylation pattern of the 5-HTTLPR. In addition it was reported that Major Depressive Disorder (MDD) patients with a short case history have lower 5-HTT promoter methylation that is associated with decreased amygdala reactivity, which might generate a more stress-adaptive epigenetic process and result

in a possibly endogenous antidepressant-like effects (Schneider et al. 2017). Likewise the correlation between severity of depression and 5-HTT reduction in the amygdala has already been shown in a meta-analysis (Gryglewski et al. 2014). In addition, some epigenetic studies have demonstrated that genotypes affect the methylation patterns (Philibert et al. 2007) as well as interactions of genotypes with other factors such as sex abuse (Vijayendran et al. 2012) or maternal prenatal stress (Wankerl et al. 2014). Given the heterogeneous susceptibility to stress-related disorders in humans (Galea et al. 2005), improved understanding of "how epigenetically modified genes are associated with adaptive stress responses, psychiatric disorders and neurobiological changes could have tremendous implications for therapeutic interventions" (Schneider et al. 2017). Therefore, it is no wonder that studies in the last years have presented more and more on sex differences in combination with methylation patterns (McCarthy & Nugent 2015). Auger and colleagues have investigated the inhibition of methyl CpG binding protein 2 (MeCP2), to demonstrate important sex differences. The social play behavior controlled by the amygdala has a higher expression level of MeCP2 in juvenile males than females (Auger & Olesen 2009). In the study of Tuominen et al. (2017), they utilized the association between neuroticism in females and males and found opposite influence levels of thalamic serotonin transporter. For example, Philibert and colleagues highlighted that female have higher CpG methylation and lower mRNA production compared to male (Philibert et al. 2008). Other studies pointed out the importance of steroid receptors and their formability at the epigenetic level (Champagne 2008; Curley et al. 2011).

1.5 DNA polymorphism

In addition to the influence of methylation patterns, prominent polymorphisms are also in the field of interest. Among other objectives, this study aims to find an effect of a certain single nucleotide polymorphism (SNP), a DNA sequence variation which occurs commonly (>1%) in a population (Brookes 2007). A SNP is a single nucleotide (A, T, C, G) within the genome which differs among the members of a given population (Yoo et al. 2014).

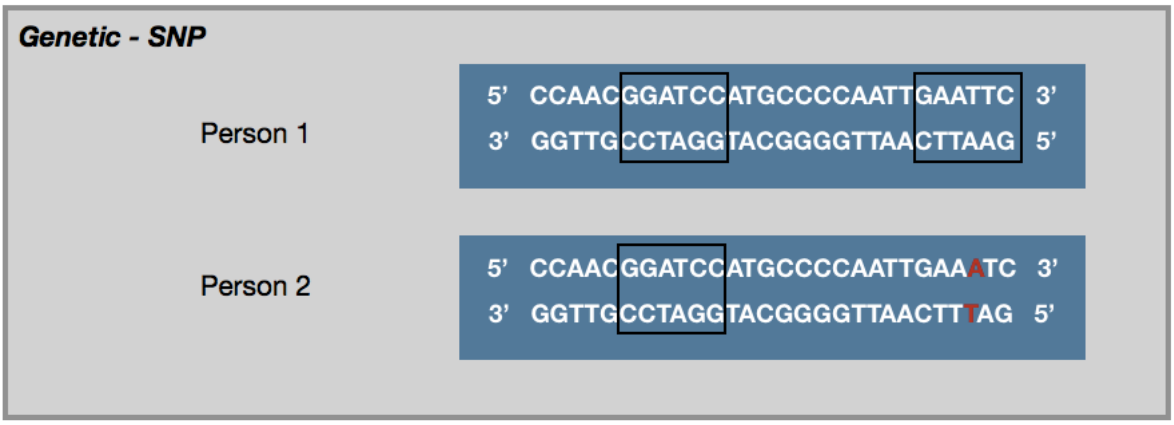

Figure 7: Single nucleotide polymorphism. One single nucleotide is changed in Person 2 (A-T) and this polymorphism can result in important functional chances. Thereby person 1 creates two fragments and person 2 one fragment. An individual difference can be displayed.

This mechanism can affect the prevalence of human diseases and therefore the importance in biomedical research of personalized medicine has greatly increased in the recent years (Bruce, 2008). It would be suggest to use SNPs as high-resolution markers related to diseases or normal traits. Researchers suggest that SNPs could be useful as genetic markers, because of their quantity and the constant inheritance over a long time (Thomas et al. 2011). Furthermore it was shown that Attention Deficit Hyperactivity Disorder (ADHD) patients have genotype dependent differences on binding potential (BP) in thalamus and cerebellum compared to healthy subjects (Sigurdardottir et al. 2016).

Based on the wide range of the serotonergic neurotransmission with behavior, sensory processing and neuropsychiatric illnesses, 5-HT is considered as a key modulatory neurotransmitter. This is particularly true for the 5-HT transporter protein which is encoded by a single gene, Solute Carrier Family 6 Member 4 (SLC6A4),

whose contribution was explored to detect individual differences in personality traits (Lesch et al. 1996). Transcriptional activity of SLC6A4 is regulated by allelic variations like repetitive sequence in 5-HTTLPR, which is compiled of a short and a long version (Canli & Lesch 2007), see figure 8. The short allele (S) with 14 repeats or the long allele (L) with up to 16 repeats of 20 – 23 base pair sequences (Heils et al. 1996), which result in differential 5-HTT expression and function. Scientists have shown that lower levels of methylation of the S variant of 5HTTLPR predicted more unresolved loss or trauma (Van Ijzendoorn et al. 2010).

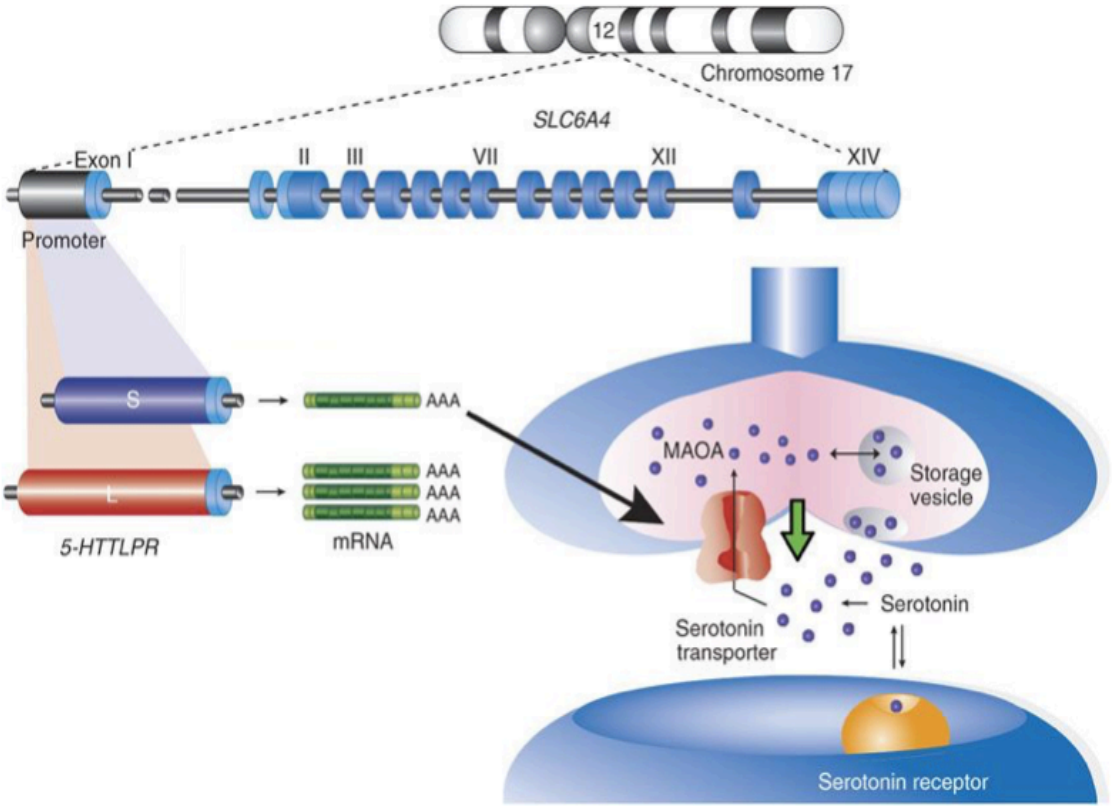

Figure 8: Allelic variation of serotonin transporter. The short (S) 5-HTTLPR variant of SLC6A4 produces less mRNA and protein, as indicated by the green arrow, than the long (L) variant (red), leading to higher concentrations of serotonin in the synaptic cleft. Taken and adapted from (Canli & Lesch 2007).

For example, a study demonstrated that a higher 5-HTT density is linked with lower synaptic serotonin levels throughout the year (Praschak-Rieder et al. 2008). The origin of central serotonergic projections show increased BP in subjects homozygous for the L allele compared with carriers of at least one S allele (Reimold et al. 2007). Sex specific disparities are also shown in imitation of aggressive models, violence and physical aggression is more common in males (Bettencourt & Miller 1996). Possible evidence for the role of genetic factors is the association between serotonin transporter gene polymorphisms and greater impulsivity in males but not in females (Manuck et al. 2000). In addition, polymorphisms in MAO-A gene are associated with antisocial personality disorder and aggression in males (Caspi et al. 2002).

Major depressive episodes (MDE) and more negative dysfunctional attitudes were affected by increased brain 5-HTT BP (Meyer et al. 2004). Other studies also show that increased 5-HTT BP correlates with mood disorders (Baldinger et al. 2014; Kraus et al. 2014). Various studies have demonstrated functional effects of SNPs on 5-HTT function, such as the association with ADHD (Bobb et al. 2005), stressful life events (Caspi et al. 2003) and depression (Kautzky et al. 2017). The risk factor for the development of psychopathic traits is indicated by long/long genotype (Glenn 2011). Furthermore, emotion related brain processes were combined with SLC6A4 variations in an fMRI study. 5-HTTLPR short variant carriers demonstrated increased amygdala activation during an emotion-related task (Hariri et al. 2002).

In the areas of neuropsychology, psychophysiology, hormones and brain imaging findings were associated with various genotypes and characteristics. Just a few potential genes have been studied for transsexualism. A recent study focused on the polymorphism in 5-alpha reductase and describe no association in MtF and FtM transsexuals (Bentz et al. 2007). On the other hand the same group showed, despite a small sample size, an association between a SNP in CYP17 gene in FtM, but not MtF people (Bentz et al. 2008). This demonstrated that the allele distribution pattern was connected to FtM transgender people. Already in 1995 a study showed anomalies in the androgen receptor gene of transsexual women (Dankbar et al. 1995). 10 years later, researchers have found differences between transgender and cisgender in length of estrogen receptor repeat polymorphisms (Henningsson et al. 2005). Microarray experiments have indicated that sex-specific genes are unusually rapid change in sequences and commonly labile in pattern expression (Ellegren & Parsch 2007). However, the interaction with SERT has not been investigated yet in detail in humans in vivo.

1.6 Positron Emission Tomography (PET)

The combination of genetic methods and neuroimaging may deepen the understanding of interaction between genes and the brain's function and structure. Hence the functional neuroimaging method of positron emission tomography (PET) is chosen. The in-vivo technique is used for clinical diagnosis of neurological, neurodegenerative diseases and psychiatric disorders as well as for research and development. PET is a powerful molecular and functional imaging technique and can be used to record sectional images of human subjects. PET labels emit positrons which are the antimatter counterpart of the electron. At first, a cyclotron produces a positron emitting radionuclide (tracer), like oxygen-15 (^{15}O), carbon-11 (^{11}C) or fluorine-18 (^{18}F), rubidium-82 (^{82}Rb) or gallium (^{68}Ga). The tracer consists of two components: a pharmaceutical product that binds to a particular target or were metabolized, and second a radioactive isotope that enables the detection of the molecules. Most commonly used tracers in diagnostic and pharmacokinetic experiments in psychiatry are ^{11}C or ^{18}F-labelled tracer molecules (Zimmer 2009). The amount of radioactive labelled compound over the total substrate is called specific activity (unit as MBq/µg). To enable detection, the amount needs to be in a particular concentration. After intravenous injection of the tracer, the targeted area will be reached. There, the emitted positrons collide with electrons in the tissue. Thereby two gamma rays are generated and emitted at an almost perfect 180° angel, each with 511 KeV energy (Fig. 9). This phenomenon is known as beta decay (β^+).

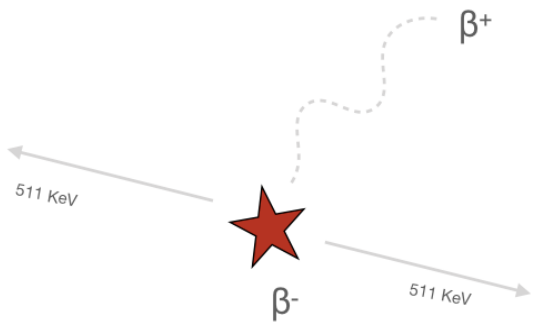

Figure 9: Beta-decay. A radioactive decay in which an atomic nucleus with emitting a positron and an electron neutrino. The two gamma rays are generated each 511 KeV in an 180° angel.

An atomic nucleus converts into a nucleus with atomic number decreased by one, while emitting an electron neutrino and a positron (Kónya & Nagy 2012). β^+ decay usually appear in proton-rich nuclei and can be considered as the decay of a proton inside the nucleus to a neutron. Compared with X-rays, gamma-rays have much higher energy and a bigger biological hazard.

$$p \rightarrow n + e^+ + v^e$$

The energy of isotopes differs in positron emission by 1mm (^{18}F) and 3mm (^{15}O) or between, such as ^{11}C (Turkheimer et al. 2014). Neuronal activity can be quantified by regional distribution of the tracer in relative blood flow in the brain or receptor binding in diverse neurotransmitter systems. The density of the gamma radiation depends on the blood flow strength, which is correlated with the neuronal activity. In PET, the location of annihilation and not the location of beta decay is included in the measurement process (Schicha & Schober 2013). From this, a three-dimensional activity image of the brain can be calculated. The scanner can record the rays by particular detectors, multiple rings of scintillation crystals. Via interaction with scintillating material, the high-energy photons are converted into visible light (Nicoletti et al. 2007). PET scanner monitor the radioactivity distribution as a function of time, temporal resolution (30s) and spatial resolution of about 2 to 8mm (Schwaiger & Pirich 2000). With different radiotracers, particular patterns of interactions of specialized brain regions and on a systems level can be illustrated. The spatial resolution is in the millimeter range, but creating a meaningful single PET image (frame) takes 40 to 90 seconds (Dudel et al. 2002) in some cases, however the imaging duration of most PET scans is about 10-90 minutes including 1 to 30 frames and more. PET allows to quantify parameters related to binding site density or radiolabeling of drug molecules to study their distribution and pharmacokinetics (Fowler et al. 1999). The images may not provide a representation of the anatomy of the examined brain and are therefore frequently combined with X-ray tomography or nuclear magnetic resonance spectroscopy 3D representations. Certainly, the field of receptor-ligand kinetics is important. The measurement of disposability, affinity and density of neuroreceptors is called binding potential (Gjedde et al. 2011). B_{max} is the total concentration of receptors in a sample of tissue. K_D is the (radioligand) equilibrium dissociation constant. BP is the ratio of B_{max} to K_D, as defined by Mintun (1984).

$$BP = \text{receptor density} \times \text{affinity} = B_{max} / K_D$$

In parallel arterial blood data are sampled from the periphery in the quantification based on radioligands not applicable to reference tissue models. Afterwards plasma will be separated from red cells by centrifugation. The plasma is further processed with a high-performance liquid chromatography (HPLC) that isolates metabolites and quantify the exact concentration of the tracer. The plasma fraction is further analyzed of bound plasma proteins. Hence, blood measurements corrected for true tracer concentrations at the entry of the organ of interest.

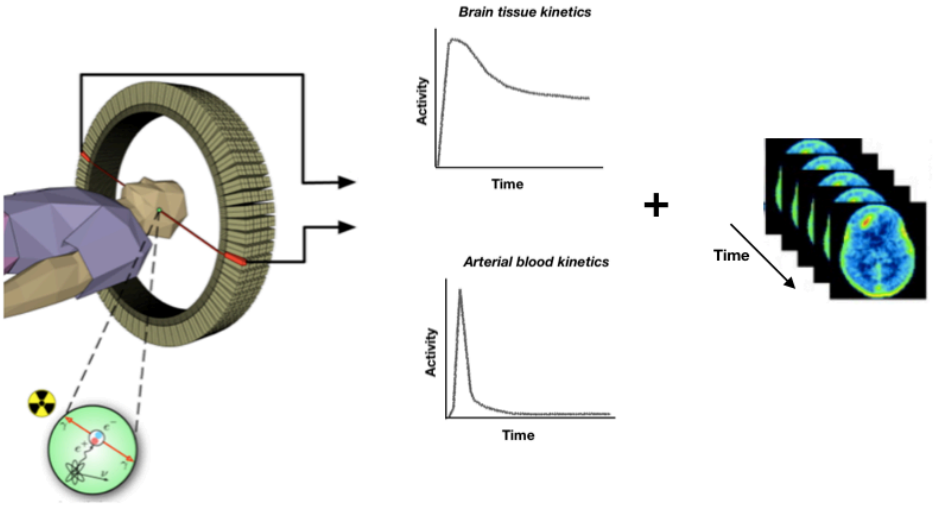

Figure 10: Process of Positron-Emission-Tomography. A positron produced by decay of a radionuclide hits an electron, both are destroyed. Two photons (gamma rays) are formed, which distance each other at an angle of almost 180°. This destruction radiation hits two detectors simultaneously. This allows the location of the positron emission to be determined mathematically using a computer. In addition, the peak is determined by blood samples and sectional images of the brain are taken over time.

PET is a detection system which measures the coincidence, accuracy physical corrections and detailed algorithm for image reconstruction allow the quantification of tracer concentrations in blood.

1.7 Aims and Relevance

So far, most societies over the world have a predominant opinion and beliefs about women and men. Whereby gender-specific prejudices and stereotypes have persisted for generations and discriminatory actions against transsexual people cannot be alleviated. The knowledge about sex differentiation has occupied for more than five decades (Wizemann & Pardue 2001). Nevertheless, there has been no significant progress in clinical application or medical health care. Transsexuals have often a long life of suffering and have to overcome many obstacles in order to arrive at the desired gender. The Standards of Care (SOC) for the health of people with gender incongruence has been a growing field for the past years. There is emerging attention on an international level in evidence-based care, education, research and public policy for transgender health. This leads to a continuing discussion and has changed the public perception that will result in new diagnostic nomenclatures in the DSM (Cohen-Kettenis & Pfäfflin 2010) and ICD. It is important to mention that transgender people are still regarded with high social incomprehension and widespread transphobic attitude. Nowadays one-third of transsexual people have experienced discrimination in health care settings (Rodriguez et al. 2018). To combat the bias and inequity, it is crucial to understand the underlying mechanisms. Indeed the main target of research has been hormones and their effects on the brain (Berenbaum & Beltz 2011) with less focus on the genetic influence. An outstanding and multidisciplinary research is necessary in order to drive scientific and therapeutic discovery for all gender. The results of this study can help to integrate neuroscientific results in best practices for gender-based health care. This could make an important contribution to the field of gender research and leverage to establish specific guidance. Furthermore, sex differences are often ignored in study design and analysis. Basically, the study will provide a better understanding of neuronal sex differences in humans and illuminate correlates of the known gender differences in the prevalence of neuropsychiatric illness. There is a lack of knowledge in the epigenetic component of the multifactorial complex of gender identity (Polderman et al. 2018). However, after years of increasing research on the basis of sex, we still do not have a clear picture of the specific epigenetic aspects of sex. Neuroimaging techniques linked with genetic methods will be able to provide even greater information of potential influencing factors in the individual's surroundings. The aim is to find correlations between genetic pattern and brain function in cis- and

transgender subjects. Results will supply information on the serotonergic role in the brain, microstructure and functional connectivity and may lead to a more comprehensive understanding of how genetic factors may influence sex and gender. Moreover, the identification of methylation pattern or functional polymorphisms that affect SERT are relevant investigations for a better understanding of unknown factors related to transsexuality and may help in health care and against discrimination. To quantify typical polymorphisms and expression patterns we will compare female and male control individuals as well as FtM and MtF transsexuals measured by PET. The combination of genetic methods and neuroimaging may deepen the understanding of interaction between genes and the brain's function and structure in transgender people. Thereby it is important to understand fundamental mechanisms and differences in epigenetics between sexes. This may extend our current knowledge about the heritable factor behind sex and transsexual people, clarify gender prejudice and hence the reduction of personal suffering.

2. Hypotheses

Genetic (MC, FC, MtF, FtM):

1. Examine the effect of SERT genotype on SERT binding potentials between those four groups

Epigenetic (females, males):

1. Examine potential differences of DNA methylation patterns between the sexes.

2. Investigate the long/short polymorphism of the SERT gene and its potential effect on methylation patterns.

3. Investigate interaction effects of genotype and sex on methylation patterns.

4. Examine the association between in vivo SERT binding and SERT methylation patterns.

5. Any potential effects of sex, genotype and methylation on SERT binding will be tested.

3. Methods

3.1. Study funding

Blood and PET data for the current investigation were used from two different studies of our group:

- Information about healthy control men and women were obtained by the study "*The Serotonin Transporter in Attention Deficit Hyperactivity Disorder investigated with Positron Emission Tomography*" (EK 784/2009) founded by the Austrian National Bank, Jubiläumsfonds Project # 13675 (Principal Investigator: PD Mag. Dr. Markus Mitterhauser, Co-Investigator: Assoc. Prof. PD Dr. Rupert Lanzenberger).

- Data from people with gender incongruence were collected by the study "*The Influence of Sex Steroid Hormones on Serotonin Transporter Binding in the Human Brain investigated by PET*" (EK 620/2008) financed by the Austrian National Bank, Jubiläumsfonds Project # 13214 (Principal Investigator: Assoc. Prof. PD Dr. Rupert Lanzenberger).

The studies were conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Medical University of Vienna. It was in cooperation with the Department of Nuclear Medicine (PET measurements and radioligand synthesis) and the Department of Psychiatry and Psychotherapy at the Medical University of Vienna, Austria.

3.2. Subjects (recruitment and procedure)

Advertisements and flyers were placed at dedicated message boards at the University of Vienna, the Medical University of Vienna, other Austrian universities, doctor's practices, local pharmacies and supermarkets. Potential participants were pre-screening on phone at first, afterwards they had to visit the psychiatry department for an integral physical and mental check. Thereby all subjects underwent standard medical examinations, electrocardiography (ECG), routine laboratory tests, were

interviewed using the Structural Clinical Interview (SCID) for DSM-IV, drug screening and a pregnancy test (in case of female subjects) to suspend any physical, psychiatric and neurological disorders. Participants with gender incongruence were recruited by the Department of Obstetrics and Gynecology, Unit for Gender Identity Disorder (Dr. Ulrike Kaufmann, MD) at the Medical University of Vienna. Diagnostic assessment of transsexualism was made after semi-structured interviews followed by DSM-IV-TR and ICD-10. All subjects had to read and sign an informed consent form after given a complete description of the study. The procedures were the same for all participants: First a screening visit, which was followed by two PET scans with different time intervals and was concluded with a follow-up examination.

3.2.1. Inclusion and exclusion criteria for controls subjects (cisgender)

Inclusion:

• Willingness and competence to sign the informed consent form
• Generally in good health based on medical history, physical examination and the structured clinical interview for DSM-IV (SCID)
• Age 18 to 55 years
• Non-smoker, no drug abuse and at most a moderate alcohol drinker

Exclusion:

• Any internal, psychiatric or neurological illness
• Current or former substance abuse
• Failure to comply with the study protocol or to follow the instructions of the investigating team
• Non-Caucasian
• Pregnancy or breast feeding (in case of women)
• Participation in studies with PET or SPECT within the last 10 years

3.2.2. Inclusion and exclusion criteria for transsexual subjects

Inclusion:

• Willingness and competence to sign the informed consent form
• DSM-IV diagnosis of Gender Identity Disorder (DSM-IV: 302.85; ICD-10: F64.0) by a clinical interview (SCID)
• General physical health based on history, physical examination, ECG, laboratory screening
• Age 18 to 50 years

Exclusion:

• Severe neurological or internal disease
• Abnormal results in routine laboratory screening or general physical examination, severe claustrophobia
• Chronic or continuous medication intake
• Steroid hormone treatment within 2 months of inclusion (including hormonal contraception and phytohormones)
• Treatment with psychopharmacological medication
• Current drug abuse (determined using a urine drug screening test at the screening visit)
• Pregnancy or breast feeding (in case of women)

3.3. PET measurement and imaging

All PET scans were conducted in a GE Advance PET tomography scanner (General Electric Medical Systems, Milwaukee, WI, USA) at the Department of Nuclear Medicine, Medical University of Vienna as in other previous studies from our group. The radioactive tracers were established, synthesized and controlled by the Department of Nuclear Medicine. For radiotracer preparation and radiochemical variables, see (Lanzenberger et al. 2012; Haeusler et al. 2009). A 5 min transmission scan was done using retractable ^{68}Ge rod sources for tissue attenuation correction (Lanzenberger et al. 2009; Hahn et al. 2012). Emission scans began with an

intravenous bolus injection of [^{11}C]-DASB with 4.7 MBq/kg body weight (Ginovart et al. 2006), the data acquisition started with measuring in 50 consecutive time frames the brain radioactivity. Total measurement time was 90 minutes and scans were acquired in 3D-mode. The emission data was corrected using 35 contiguous slices (matrix 128*128) using an iterative filtered back projection algorithm (FORE-ITER) with a spatial resolution of 4.36 mm full-width at half maximum 1 cm next to the center of the field of view (Lanzenberger et al. 2009). To minimize misalignment inherent to the normalization procedure the template was created from T1-weighted images (Kranz et.al 2014). The template was spatially normalized using the SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK) used in the co-registration step. After co-registration to the MRI scans, the transformation matrix was then applied to the summed PET images. The SERT BP_{ND} was quantified using the multilinear reference tissue model (MRTM2) as described from Ichise (2003). Individual summed PET images where spatially normalized to a custom symmetrical PET template using SPM12. Finally, a tracer-specific template was flipped and averaged with the unflipped template (Takao et al. 2011). Whole-brain voxel-wise SERT BP_{ND} maps were computed and the cerebellum region was used as a reference region.

3.4. Investigated SNPs

We examined the SNP rs6311, a SNP located upstream of serotonin receptor 2A (HTR2A). The minor allele of rs6311 has been shown to reduce the expression of 5' untranslated region of HTR2A mRNA. Several scientific studies have investigated the link between rs6311 and disorders like panic disorder (Unschuld 2007), major depression (Lin 2009) or autism spectrum disorder (Guhathakurta 2009). We also investigated the serotonin transporter gene (SLC6A4) which contains a length polymorphism that consists of 44 base pair insertion/deletion and results in two common alleles, the long allele (L) and the short allele (S) (Tartter and Ray, 2011).

3.5. Blood analysis

Procedures were elaborated as previously described by our group (Baldinger et al. 2014). Blood samples from each participant were drawn in 9 ml Ethylene diamine tetraacetic acid (EDTA) tubes and DNA was isolated from whole blood using the QiaAmp DNA blood maxi kit (Qiagen, Hilden, Germany). The procedure followed the protocol ‘‘DNA Purification from Blood or Blood Fluids’’ of the QIAamp DNA Mini and Blood Mini Handbook 11/2007 (QIAGENÒ, Hilden, Germany). Genotyping was executed using the iPLEX assay on the MassARRAY MALDI-TOF mass spectrometer as described by Oeth et. al (2007). Allele specific extension products were identified and genotypes allocated by Typer 3.4 Software (Sequenom, San Diego, CA). All applied quality criteria were appropriate [individual call rate >80%, SNP call rate >99%, identity of genotyped of CEU trios (Coriell Institute for Medical research, Camden, NJ) with HapMap database >99%]. Protocol of the DNA methylation design is described by Suchiman et al (Suchiman 2015). Shortly described, 100 ng of genomic DNA was bilsulfite converted using the EZ-96 methylation kit, Shallow-Well Format (ZYMO Research, USA), followed by PCR amplification. Step down PCR reaction was done as per protocol. After cleaving the products into smaller fragments and removal of excess ions, the samples were analyzed with the Epityper 1.2 (Agena Bioscience, Germany) and pre-processed using the EpiTYPER Analyser. Quality criteria included: sample call rate >50% CpG callrate >85% and duplicate values with standard deviation <0.1.

3.6. Regions of interest (ROIs)

The selection of standardized regions of interest (ROIs) was based on regions with high expression of the SERT. Those regions included the anterior cingulate cortex (ACC), amygdala, caudate, dorsal raphe nucleus (DRN), hippocampus, hypothalamus, insula, medial cingulate cortex (MCC), medial raphe nuclei (MRN), putamen and the thalamus. The Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002) (52 ROIs) was used for the extraction of binding potential. SERT BP_{ND} in this 11 MRI-based brain ROIs were compared in high-resolution PET images. ROI SERT nondisplaceable binding potential (BP_{ND}) was taken from the Hammer Maximum Probability Atlas (Hammers, et al., 2003).

Resulting transformation matrices were used to coregistered parametric images into Montreal Neurological Institute (MNI) T1 single-participant brain (Tzourio-Mazoyer et al. 2002). Parametric voxel-wise maps were created by specifing the BP_{ND} for each voxel and lastly averaging brain maps within the groups (FC, MC, MtF, FtM).

3.7. PET processing

Microsoft Excel 2010 for simple calculations and polar charts and SPM12 for parametric voxel-wise analysis were used for statistical analyses. PET images were visually examined and motion-corrected using SPM12, and then normalized to a tracer-specific template to enable automated ROI analysis. Time activity curves were obtained by applying a ROI-template, defined from Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al. 2002) to each scan via MATLAB program (The MathWorks Inc. R2018a, US). The kinetic modeling tool PKIN implemented in PMOD and the “multilinear reference tissue model” (MRTM/MRTM2) were used to calculate BP_{ND} (Ichise et. al, 2003).

3.8. Statistics

SPSS version 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) was used for descriptive statistics, diagrams, correlations and regressions. The significance level was set at $p<0.05$ and corrected for multiple comparisons using the Benjamini-Hochberg correction. All p-values were ranked from the smallest to the largest one and after correction the value below 0.05 represents significance. To examine expected differences between sexes, analyses were run separately for the four groups (FC, MC, FtM, MtF). Descriptive parameters were calculated and SERT methylation levels were tested for normality using the Shapiro-Wilk test. In case of deviation from normality, Mann-Whitney was computed to test for differences between study groups. Effects of group on methylation levels were tested by using linear mixed model using the average mean of methylation levels from each region as well as individual CpG sites methylation values as the dependent variables. The model tested for main effects and any possible interactions

between group and CpG sites on methylation. If executed significant, post hoc analysis included Mann-Whitney tests and t-test in case of normality. Effects of SNPs on methylation levels were also tested for using genotype (major vs. minor allele) and group (transsexual vs. controls) as fixed factors and methylation levels as the dependent variable. Effects on SERT BP_{ND} were examined with linear mixed models for each SNP, using the genotype (major vs minor allele) and group (transsexual vs. controls) as fixed factors and ROI as repeated factor and SERT BP_{NP} as the dependent variable. Other effects (e.g., for age) were also tested for and were excluded if insignificant.

4. Results

In the first part of this study a total number of 52 subjects were included, aged 18-54 years, consisting of 11 female controls, 15 male controls, 12 female-to-male and 14 male-to-female transsexuals. The age range was comparable between groups. The mean age and standard deviation (± SD) of the healthy controls were in FC = 30 ± 9.60 and in MC = 34 ± 10.74. The transsexual group had a mean age of FtM = 26.8 ± 6.5; MtF = 29.2 ± 7.9.

For the second part of the study, the analysis of DNA methylation, a total number of 90 subjects were investigated, including 38 males (age = 28.6 ± 7.9) and 52 females (age = 27.2 ± 6.9).

4.1. Effects of SERT short/long allele on BP in MC, FC, MtF and FtM

Results revealed a main effect of genotype ($F_{3.22}$ = 39, p<0.005, uncorrected) and an interaction effect of genotype and group ($F_{1.70}$ = 39, p<0.05, uncorrected). Post hoc testing for the comparison of the four groups (FC, MC, MtF, FtM) indicated a genotype dependent difference in SERT binding potential (BP_{ND}) within and between groups:

For the MtF group a higher SERT BP_{ND} was found for the long allele in the cingulate cortices ($F_{10.21}$ = 11, p<0.01, uncorrected, ACC), ($F_{6.08}$ = 11, p<0.05, uncorrected, MCC), ($F_{4.73}$ = 11, p<0.05, uncorrected, PCC) as well as thalamus ($F_{11.42}$ = 11, p<0.01, uncorrected) and putamen ($F_{15.11}$ = 11, p<0.01, uncorrected). No other genotype dependent differences were found for any other group.

Comparing the MtF and FtM groups, a higher SERT BP_{ND} was found for the long allele carriers in the MtF group in the amygdala (t = -2.60, = p<0.05, uncorrected), putamen (t = -2.80, p<0.05, uncorrected), caudate (t = -2.94, p<0.05, uncorrected), midbrain (t = -2.41, p<0.05, uncorrected) and the medial raphe nuclei (MRN) (t = -2.27, p<0.05, uncorrected).

While comparing MtF vs MC, those carrying the short allele had lower BP_{ND} compared to MC group in the anterior (t = -3.16, p<0.01, uncorrected) and medial cingulate cortices (t = -2.42, p<0.05, uncorrected) as well as the insula (t = -2.29, p<0.05, uncorrected).

Comparing FtM vs FC carrying the short allele, higher BP_{ND} was detected in the latter group in the amygdala ($t = -2.48$, $p<0.05$, uncorrected), putamen ($t = -2.83$, $p<0.05$, uncorrected) and dorsal raphe nuclei (DRN) ($t = -2.28$, $p<0.05$, uncorrected).

Comparing FtM to MC, differences were detected for the short allele carriers as higher BP_{ND} was found for MC in the amygdala ($t = -3.15$, $p<0.01$, uncorrected), caudate ($t = -2.83$, $p<0.05$, uncorrected), putamen ($t = -2.77$, $p<0.05$, uncorrected) and hypothalamus ($t = -3.20$, $p<0.01$, uncorrected). All p-values given are uncorrected as they did not survive multiple corrections. The larges differences in SERT BP_{ND} between major and minor allele were seen for the four groups in caudate, ACC, MCC and thalamus (see figures 11 to 14).

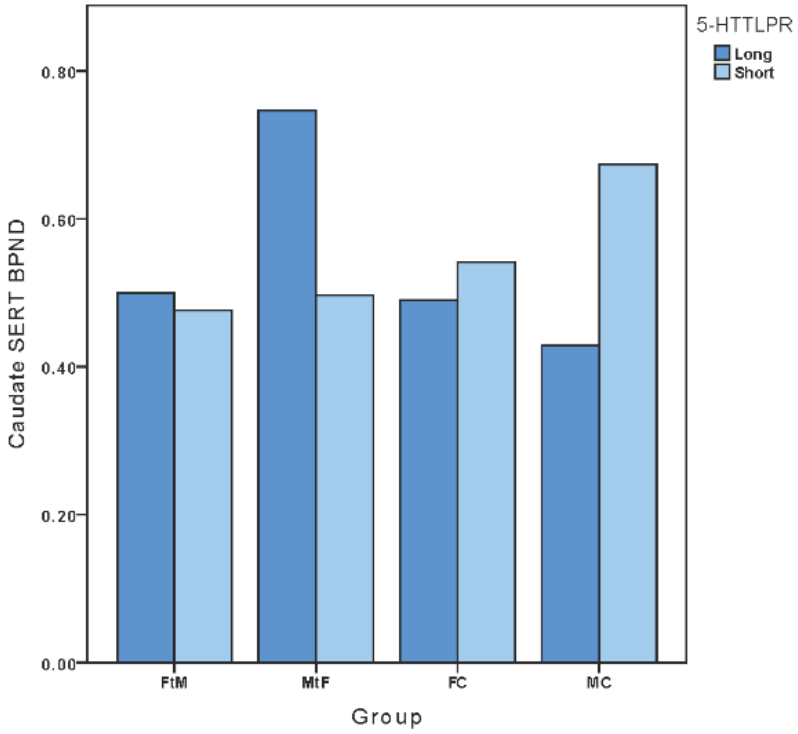

Figure 11: SERT BP_{ND} in caudate nucleus for all four groups (FtM, MtF, FC, MC). The serotonin transporter binding potential (SERT BP_{ND}) in the caudate for female-to-male (FtM), male-to-female (MtF), female control (FC) and male control (MC). The dark blue depicts the major allele while the light blue indicates the minor allele. A larger bar indicate a higher SERT BP_{ND} for this group in the caudate nucleus.

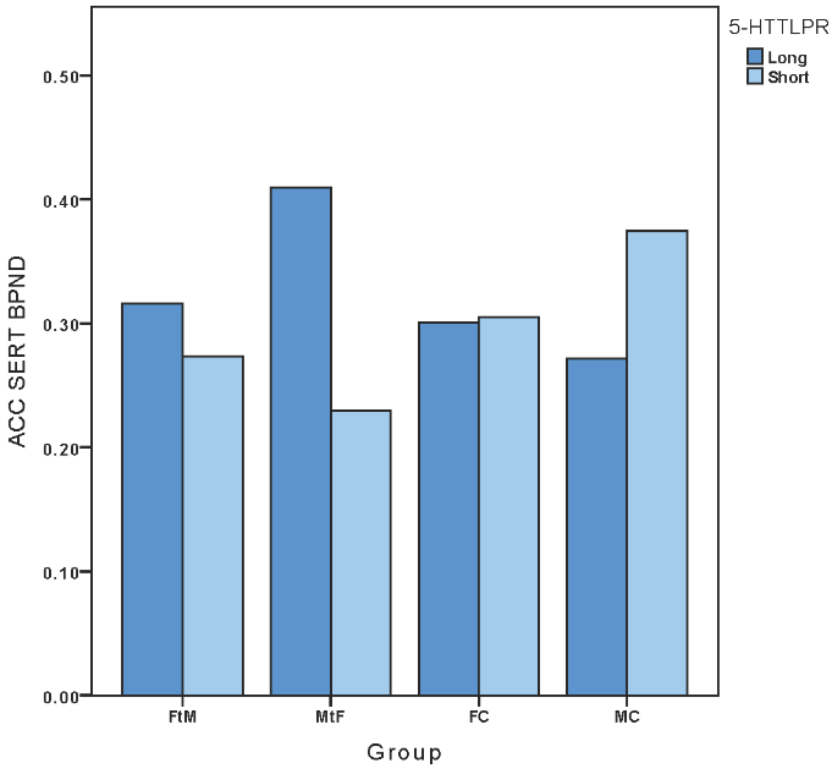

Figure 12: SERT BP_{ND} in anterior cingulate cortex (ACC) for all four groups (FtM, MtF, FC, MC). The serotonin transporter binding potential (SERT BP_{ND}) in the anterior singular cortex (ACC) for female-to-male (FtM), male-to-female (MtF), female control (FC) and male control (MC). The dark blue depicts the major allele while the light blue indicates the minor allele. A larger bar indicates a higher SERT BP_{ND} for this group in the ACC.

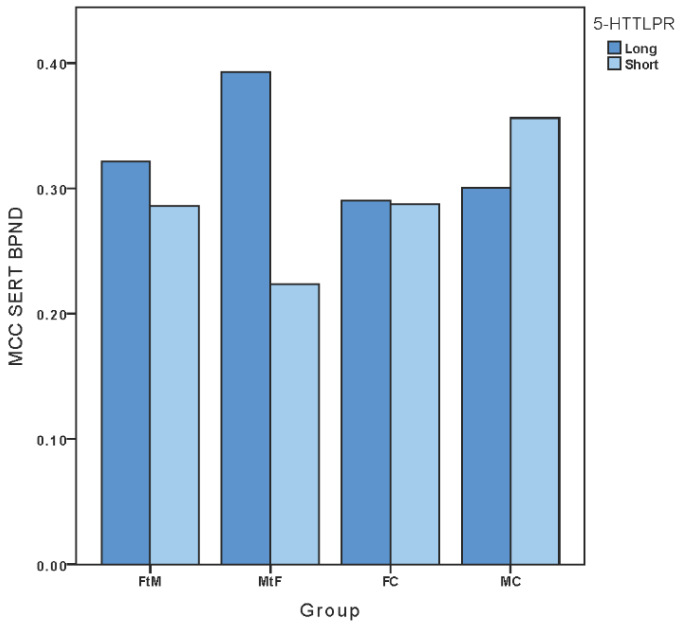

Figure 13: SERT BP_{ND} in the midcingulate cortex (MCC) for all four groups (FtM, MtF, FC, MC). The serotonin transporter binding potential (SERT BP_{ND}) in the medial cingulate nucleus (MCC) for female-to-male (FtM), male-to-female (MtF), female control (FC) and male control (MC). The dark blue depicts the major allele while the light blue indicates the minor allele. A larger bar indicate a higher SERT BP_{ND} for this group in the MCC.

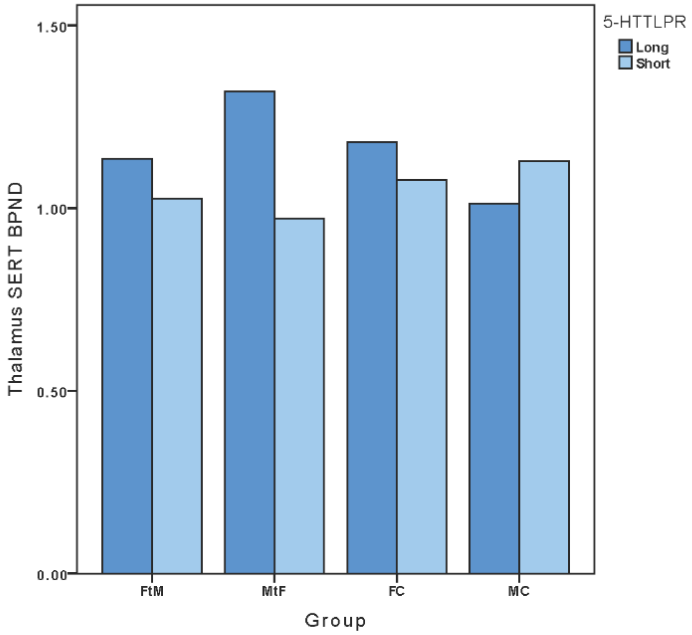

Figure 14: SERT BP_{ND} in thalamus for all four groups (FtM, MtF, FC, MC). The serotonin transporter binding potential (SERT BP_{ND}) in the thalamus for female-to-male (FtM), male-to-female (MtF), female control (FC) and male control (MC). The dark blue depicts the major allele while the light blue indicates the minor allele. A larger bar indicate a higher SERT BP_{ND} for this group in the thalamus.

The figure 15 shows the PET images of all four groups (FtM=F2M, MtF=M2F, FC, MC). The color of a frame indicates the corresponding section of SERT BP_{ND} in thalamus. A significant difference in MtF transsexuals can be seen in thalamus, compared to the other three groups (FtM, FC and MC). The figure shows triplanar structural images and superimposed distribution maps of SERT binding using [^{11}C]DASB.

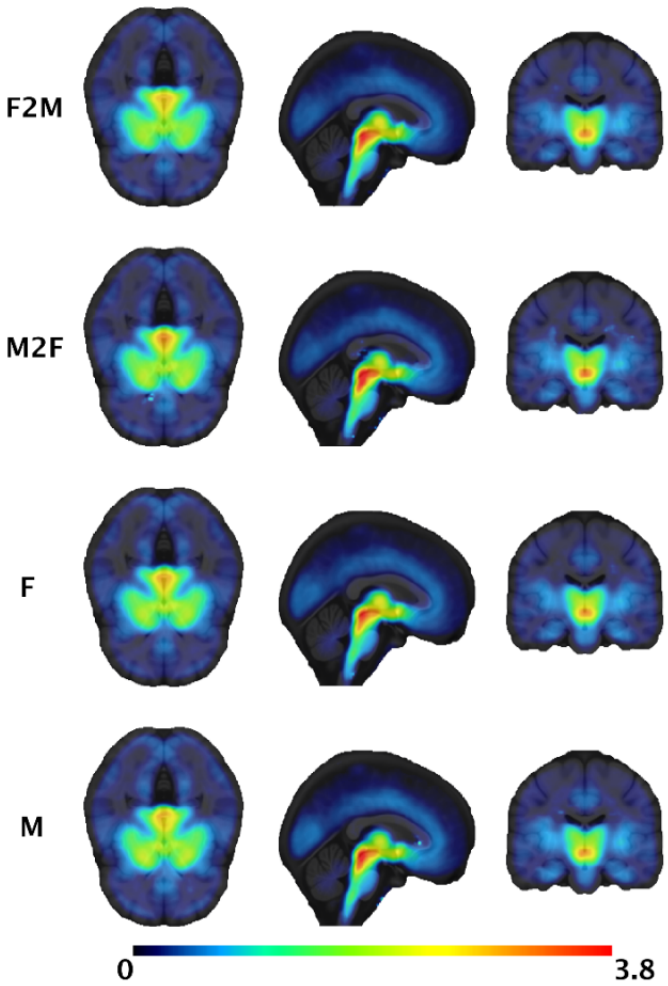

Figure 15: Serotonin distribution. The color in the figure is showing the distribution of SERT (in axial, sagittal, and coronal view from left to right) in all four groups (FtM, MtF, FC, MC). The color is indicating the binding potential (BP_{ND}) in different regions, from low (blue) to high BP_{ND} values (red).

4.2. DNA methylation patterns between the sexes (FC and MC)

Results revealed a difference in two CpG sites between females and males: CpG 13 ($F_{11.68}$ = 90, p<0.001, uncorrected) and CpG 31.32 ($F_{18.76}$ = 90, p<0.001, uncorrected) and show higher methylation in females compared to males, as can be seen in figure 16.

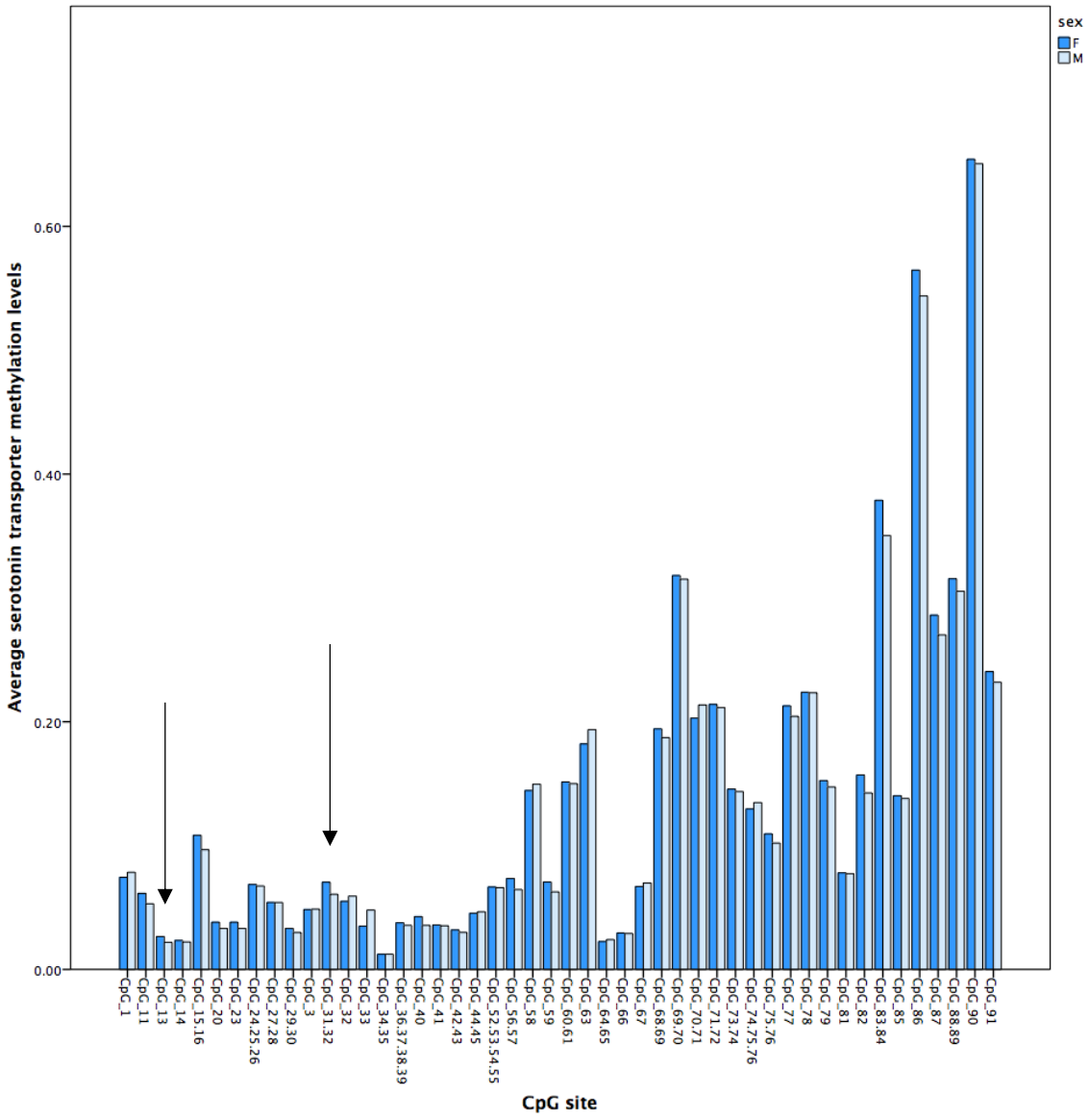

Figure 16: SERT methylation level for different CpG sites in females and males. The average serotonin transporter (SERT) methylation pattern for females (F) is depicted in dark blue and males (M) in light blue for different CpG sites.

4.3. Effects of genotypes and sex (FC and MC) on methylation patterns

Results indicated an effect of sex and genotype on methylation patterns in the sexes. Genotype effects of the SNP rs6311 was detected in females for the following CpGs (and can be seen in figure 17) sites with higher methylation levels found for the major allele (CC) compared to the minor allele carriers (CG/GG): CpG 3435 ($F_{9.20}$ = 52, p<0.001, uncorrected), CpG 52535455 ($F_{6.32}$ = 51, p<0.005, uncorrected), CpG 77 ($F_{6.36}$ = 52, p<0.005, uncorrected), CpG 78 ($F_{5.19}$ = 49, p<0.005, uncorrected), CpG 8384 ($F_{10.40}$ = 52, p<0.001, uncorrected), CpG 85 ($F_{6.06}$ = 52, p<0.005, uncorrected), CpG 8889 ($F_{6.06}$ = 52, p<0.005, uncorrected), CpG 90 ($F_{4.94}$ = 52, p<0.005, uncorrected), CpG 91 ($F_{4.64}$ = 52, p<0.005, uncorrected).

In males, genotype effects with minor allele having higher methylation compared to major allele carriers were found for one CpG site ($F_{8.27}$ = 35, p<0.001, uncorrected; CpG 82) (figure 18).

When examining effects of sex and genotype on methylation levels, higher methylation levels were detected for females carrying the major allele compared to males carrying the major allele for multiple CpG sites: CpG 7576 ($F_{5.72}$ = 21, p<0.005, uncorrected), CpG 77 ($F_{7.13}$ = 22, p<0.005, uncorrected), CpG 81 ($F_{4.57}$ = 22, p<0.005, uncorrected), CpG 82 ($F_{6.16}$ = 21, p<0.005, uncorrected), CpG 8384 ($F_{16.10}$ = 22, p<0.001, uncorrected), CpG 86 ($F_{6.67}$ = 22, p<0.005, uncorrected), CpG 87 ($F_{7.51}$ = 22, p<0.005, uncorrected), CpG 8889 ($F_{8.23}$ = 22, p<0.001, uncorrected), CpG 90 ($F_{6.22}$ = 22, p<0.005, uncorrected), CpG 91 ($F_{6.71}$ = 22, p<0.005, uncorrected).

No effect was detected between the sexes for the long polymorphism (figure 19 and 20) while a difference can be observed for the short polymorphism only. Females carrying the short allele have higher methylation than male carriers, as showed in figure 20: CpG 13 ($F_{10.72}$ = 56, p<0.001, uncorrected), CpG 1516 ($F_{4.93}$ = 55, p<0.005, uncorrected), CpG 3132 ($F_{10.39}$ = 56, p<0.001, uncorrected), CpG 363738 ($F_{7.40}$ = 56, p<0.001, uncorrected), CpG 82 ($F_{4.66}$ = 54, p<0.005, uncorrected), CpG 8384 ($F_{4.52}$ = 56, p<0.005, uncorrected).

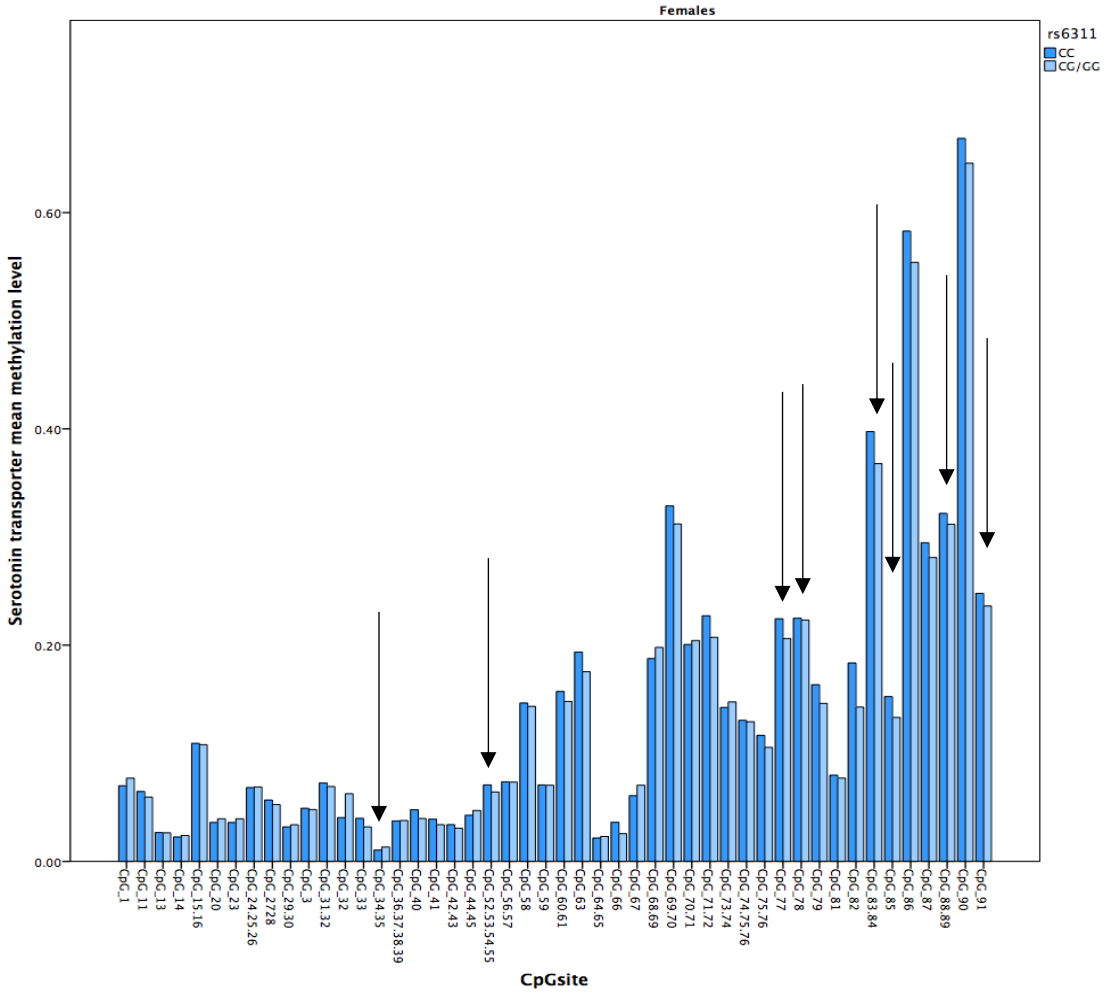

Figure 17: SERT methylation level for different CpG sites in females. The average serotonin transporter (SERT) methylation pattern depending on the SNP rs6311 for females is shown. The major allele (CC) is depicted in dark blue and the minor allele carriers (CG/GG) in light blue for different CpG sites.

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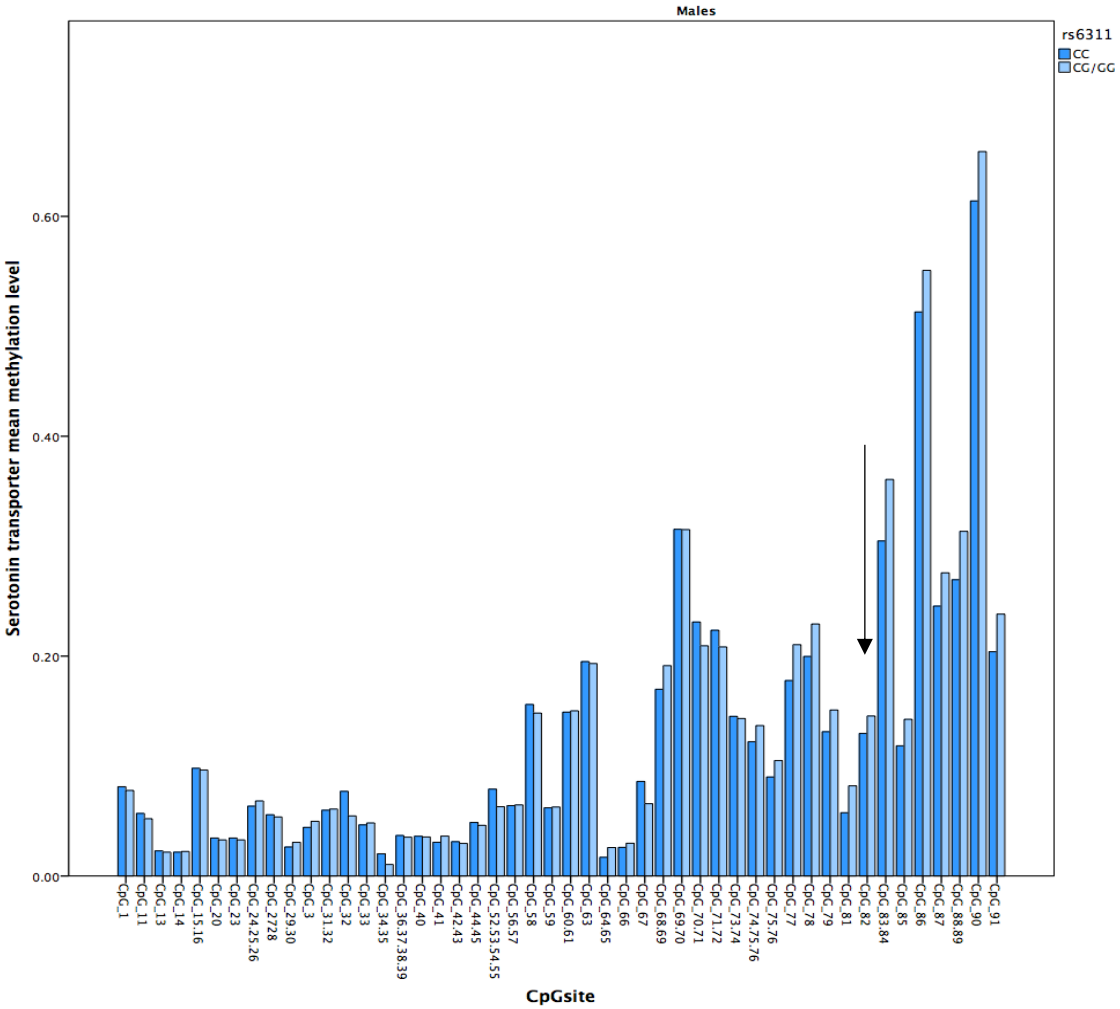

Figure 18: SERT methylation level for different CpG sites in males. The average serotonin transporter (SERT) methylation pattern depending on the SNP rs6311 for males is shown. The major allele (CC) is depicted in dark blue and the minor allele carriers (CG/GG) in light blue for different CpG sites.

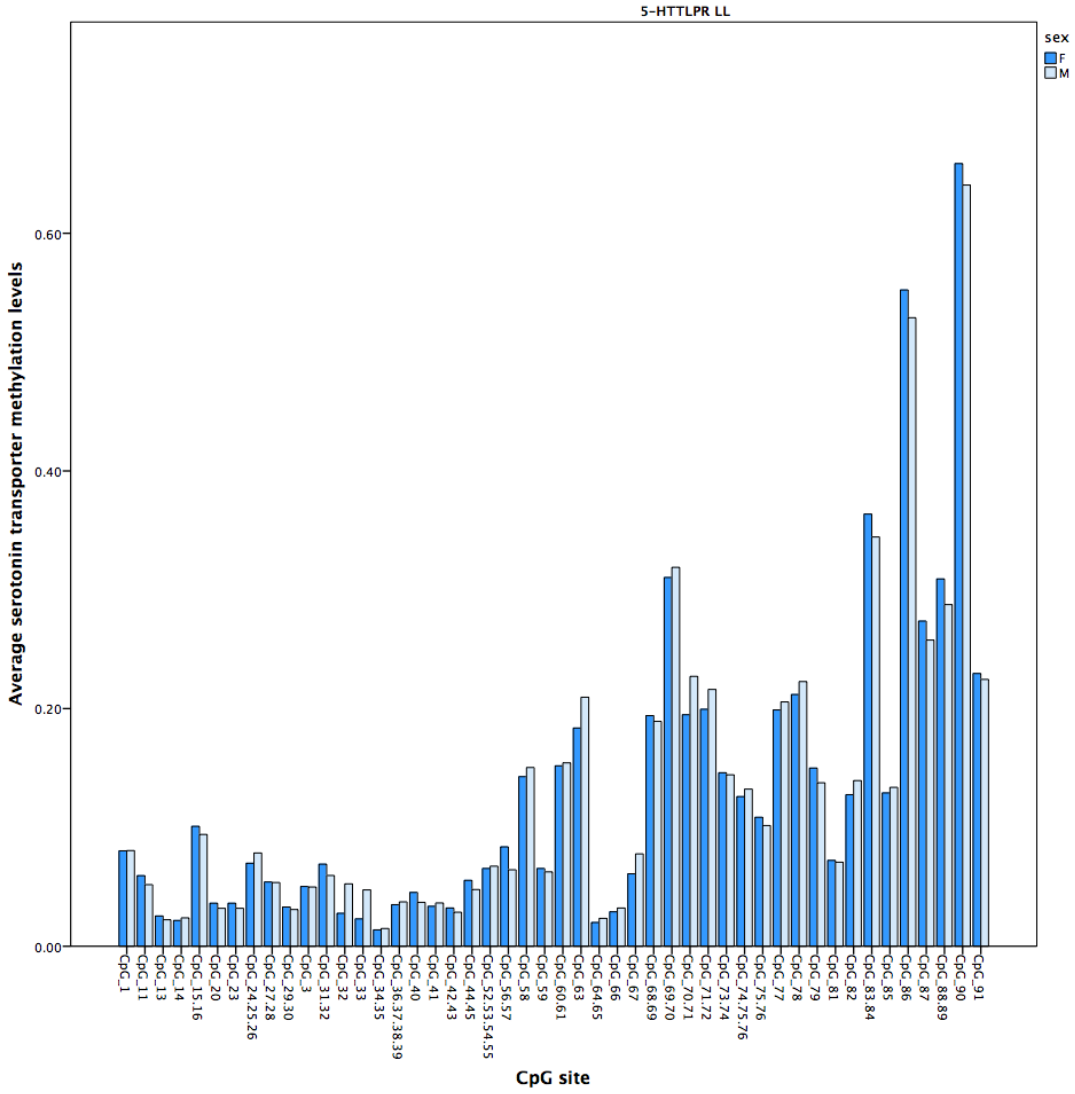

Figure 19: SERT methylation levels for CpG sites depending on the long polymorphism in the SERT gene in females and males. The average serotonin transporter (SERT) methylation pattern depending on the long polymorphism (LL) in the serotonin gene (5-HTTLPR) is shown. The methylation pattern for females (F) is depicted in dark blue and for males (M) in light blue for different CpG sites.

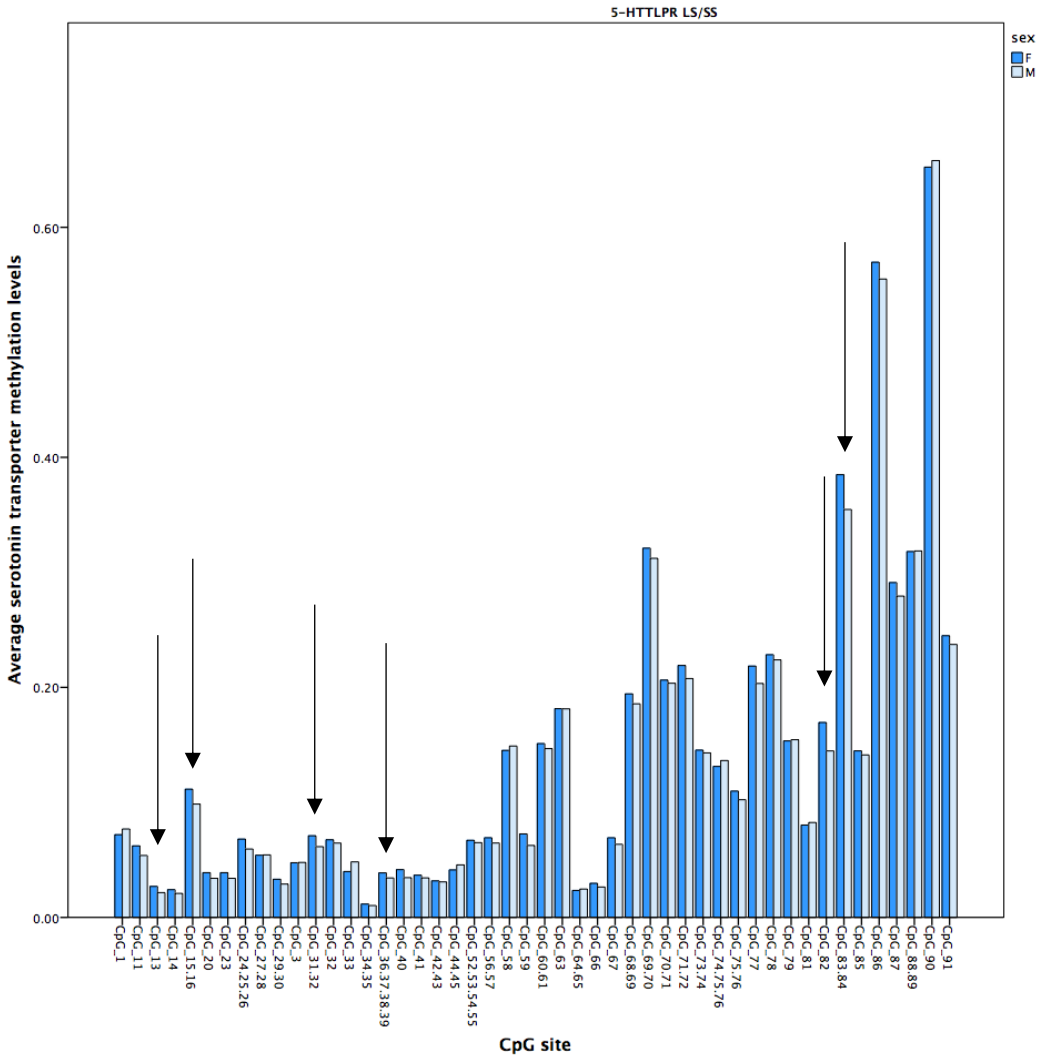

Figure 20: SERT methylation levels for CpG sites depending on the long and short polymorphism in the SERT gene in females and males. The average serotonin transporter (SERT) methylation pattern depending on the long and short (LS/SS) polymorphism in the serotonin gene (5-HTTLPR) is shown. The methylation pattern for females (F) is depicted in dark blue and for males (M) in light blue for different CpG sites.

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4.4. Association between SERT binding and methylation pattern (FC and MC)

By examining the correlation between the in vivo SERT availability and SERT DNA methylation levels, both between and within groups, no association was observed.

5. Discussion

Our investigation focused on the genetic/epigenetic patterns and brain function in subjects with gender incongruence (transsexual subjects) and control subjects (cisgender subjects). Most of the results did not survive corrections for multiple comparison and no correlation between SERT binding and methylation pattern detected. We examined serotonergic genetic variants as well as the SERT DNA methylation pattern of the SERT gene in cis- and transsexual people. A tendency of genotype effects of the SERT gene on the SERT BP_{ND} between the four groups (FtM, MtF, FC, MC) was detected before applying corrections for multiple comparisons. A few CpG sites showed differential methylation patterns between the sexes with females having higher methylation patterns compared to males. Also no correlation between blood DNA levels and in vivo SERT BP_{ND} was found.

The first part including a comparison of the effect of SERT genotype (short/long allele) on SERT binding potentials between MtF, FtM MC and FC. Our results indicate a tendency of genotype effects both within and between groups. The MtF group showed a higher SERT BP_{ND} in the long allele in the cingulate cortices, thalamus and putamen compared to short allele. The study of Spies et al. showed similar results in SERT BP_{ND} that MtF are different at baseline measurement (before hormonal medication) compared to all other groups (Spies et al. 2016). Interestingly, also the PET imaging with [^{11}C]DASB indicated a difference in the SERT BP_{ND} in MtF transsexuals in thalamus compared to the others. The results are in accordance with the findings of Jovanovic et al., where they showed a higher SERT BP in men as well as a higher serotonin receptor BP in women (Jovanovic et al. 2008). The SERT availability in MtF transsexuals before hormonal treatment is comparable to SERT BP in male controls. Another study showed no SERT asymmetry in the midcingulate in MtF transsexuals (Kranz et al. 2014), which suggest that thalamus, putamen and cingulate cortices may be more important for the masculinization in MtF people. The effect in MtF transsexuals may indicate a gender-dimorphic organization of these brain structures and a changed masculinization in MtF transsexuals related to the other groups. It should be noted that the degree of masculinization in the brain is not influenced by the degree of masculinization of genitals at birth (Swaab & Bao 2013) and changes in the course of life.

In the MtF group we found a tendency for higher SERT BP_{ND} for the long allele in the amygdala, putamen, caudate, midbrain and the medial raphe nuclei compared

to FtM while the FtM group carrying the short allele had lower BP_{ND} compared to MC group in the anterior and medial cingulate cortices and the insula. It should be mentioned that the SERT BP_{ND} in the midbrain is anyway different to amygdala and thalamus and increased in the long allele (Reimold et al. 2007). It is not possible to predict genotype effects based solely on BP_{ND} of the midbrain. In addition, our results show that comparing FtM with FC carrying the short allele, higher BP_{ND} was detected in the latter group in the amygdala, putamen and dorsal raphe nuclei. Comparing FtM to MC, differences were detected for the short allele carriers as higher BP_{ND} was found for MC in the amygdala, caudate, putamen and hypothalamus. Other studies suggest that less SERT input to the amygdala followed with lower SERT BP_{ND}, may result in increased amygdala activity (Abercrombie et al. 1998). Furthermore, numerous studies showed the link between lower BP_{ND} in the amygdala in subjects with major depressive disorder compared with healthy people (Parsey et al. 2006). Other areas are also affected as shown in different studies which investigated the influence from long/short allele of the SERT as a potential risk factor for psychopathic traits (Caspi et al. 2010; Baldwin M Way & Taylor 2010; Hariri et al. 2002). It should be noted that an altered SERT BP_{ND} in different brain regions have diverse effects of well-being as well as healthiness and is influenced by gender dimorphism, but more research in this field is needed in order to make targeted statements. Our findings can be interpreted in several ways. First, it is imaginable that the amygdala, raphe nuclei, cingulate cortices and insula are crucial structures in the pathophysiology of gender dimorphism. All these regions are linked with emotions, memory as well as inner attitudes and can therefore influence women` and men` feelings in different ways. Second, the abnormalities in other brain areas may not be specific to SERT, but perhaps may involve in other serotonergic function and therefore do not influence the gender role. Third, the total number of available SERT binding sites are different in diverse brain areas and also different in men and women. A lower level of synaptic serotonin promotes SERT internalization (Ramamoorthy et al. 2016), this could end in fewer SERT neurons or decreased expression of SERT in various brain regions. The lower level of affinity could potentially result in a higher level of expression of the serotonin protein in the raphe nuclei for example. Fourth, other SNP, which are also involved in metabolism and synthesis of steroid hormones, can be associated with gender incongruence and could have a different specificity in transsexual people. Data suggest the cytochrome P450 protein as a candidate gene of FtM

transsexualism (Coolidge et al. 2008). As discussed previously, serotonin is involved in mechanisms and expression of steroid hormones. Therefore, it is not surprising that women and men as well as FtM and MtF not only produce different levels of sex hormones, but also the availability and density of brain SERT is different. Animal studies have also showed increased SERT density in rats after administration of testosterone (Mcqueen et al. 1999). These findings are in accordance to studies in humans, which demonstrated that FtM transsexuals had increased SERT BP_{ND} in amygdala after four months of hormonal treatment (Kranz et al. 2015). That may due the fact that the amygdala of men are larger and exhibit a higher density of androgen compared to estrogen receptors (Ruigrok et al. 2014). An altered SERT BP_{ND} is therefore postulated to be related to the amount of steroid hormones. Hence, based on this findings, we suggest that the MtF group is influenced by an altered SERT BP_{ND} depending on the long allele and FtM people depending on the short allele, although the molecular basis of this finding is not clear. It remains to mention that both, allele and the amount of particular receptor are different in women and men and have impact on lifetime events. Another receptor and diverse BP_{ND} on the short vs long allele have other impact of the sex dimorphism.

In the second part of the investigation, DNA methylation levels were tested between the sexes. Two CpG sites show higher methylation in females compared to males. Additionally, genotype effects of the SNP rs6311 were detected in females for several CpG sites with higher methylation levels found for the major allele compared to the minor allele carriers. In males in contrast, genotype effects was observed with minor allele carriers having higher methylation compared to major allele carriers. This was found only for two CpG sites in the SERT gene while further sites did not survive corrections. In contrast to the findings of Philibert and colleagues, they pointed out that the CpG methylation in females was higher and thereby mRNA production lower which is associated with the short allele (Philibert et al. 2008). The serotonin synthesis seems to be different in women and men and was controversially discussed in literature. So, Nishizawa et al. found higher rates in males (Nishizawa et al. 1997) whereas Chugani and colleagues found it in females rates (Chugani et al. 1998). That could be imply that long and short allele of SERT affect different parts in the development of gender.

When testing for correlations between blood DNA methylation levels of the SERT gene and brain SERT availability no association was detected. It could be require to examine further sites, because our results do not indicate comparing blood and brain specifically for the probed sites. Beside it could be possible that the effect size was just too small to be detectable. The regulation of DNA methylation is depending on genetic variations and remains partly consistent across the lifespan (Gaunt et al. 2016). Our findings are in line with the in vivo study of Chou et al. (2013), which showed that the availability of SERT in the human brain is not correlated with the genotype of the SERT gene, which is in contrast to several in vitro findings. The availability of SERT in the human brain based on the polymorphisms of the SERT gene can be associated with blood DNA methylation, but regarding our results causal factors in the development of gender incongruence have not be found. A lot of studies showed correlations for the SERT methylation level with diseases, in most instances the attention is focused on the etiology of major depressive disorder as well as anxiety disorders, major depression, and aggression-related personality traits (Lesch et al. 1996). Some studies from European countries have suggest the important role of SERT gene in some disorders, which could not replicated in Asian studies (Kunugi et al. 1997). This discrepancy can be explained by a large multinational epidemiology and the specificity of appearance of SNPs. The heterogeneous clinical picture of gender incongruence can be considered in the same way. More investigations are necessary in brain SERT availability and methylation pattern in order to use gender differences on clinical status or treatment outcome. We assume that SERT function are influenced by both, genetic predisposition and epigenetic modifications.

6. Limitations

Firstly, the small sample size presented a limitation to our study. To detect meaningful genetic or epigenetic differences, further studies should include more subjects.

Secondly, we only investigated a several of SNPs in our study. In order to obtain meaningful results as well as get a broad view of the underlying genetic mechanism and difference between the sexes, it is important to explore several SNPs in further studies.

Thirdly, we analyzed DNA methylation of the SERT from whole blood as representative for the total brain in vivo SERT expression, however DNA methylation tends to be tissue specific (Jones & Takai 2001). Other findings show correlation between SLC6A4 DNA methylation and the brain in relation to peripheral blood (Wang et al. 2012), therefore no clear and definite conclusions are possible with investigations that only consider whole blood. Furthermore, the genetic data was extracted from lymphoblast cell lines, not serotoninergic neurons. These cell lines have transcriptional signatures reflect of their cognate donors (Philibert et al. 2007). For further research, the removal of neurons (for example from olfactory cells) would be a good consideration.

7. Conclusion

The study aimed to establish a link between neuroimaging techniques and genetic methods for a better understanding of sex differences in humans. The link makes an attractive study designs possible to answer questions raised in the field of genetics and epigenetics. The SERT distribution was obtained with PET using the highly selective tracer [^{11}C]DASB to quantify the binding potentials in different ROIs.

Despite the fact that the values don't survived correction, the tendencies provide a first hint for further studies. It is crucial to realize that innate and environmental values influence every persons surroundings in a different way. Even if it is possible to identify the exact epigenetic mechanism in transsexual people that does not mean there is less influence of genetic factors (Bandelow et al. 2016). The investigation of in vivo molecular imaging methods allow to make appealing study designs in order to answer questions in many fields of neuroscience and should be pursued further. The next steps should imply the replication and expansion of our results with similar investigations with a larger sample size group and different neurotransmitters as well as including more SNPs.

Similar to other published studies, our findings can be considered in the way that allelic variations of serotonin transporter are dependent on environmental influences (Way & Taylor 2010) and not all variables influence the outcomes in the same way (Brummelte et al. 2017). So that means that every person will be affected in a different way because of the individual surrounding. McCarthy and colleagues indicate that comprehensive knowledge of neural processes will only be achieved by unifying sex as a biological variable (McCarthy 2016).

8. References

Abercrombie, H.C. et al., 1998. Metabolic rate in the right amygdala predicts negative affect in depressed patients. *NeuroReport*, 9(14), pp.3301–3307.

Adell, A. et al., 2002. Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Research Reviews*, 39(2–3), pp.154–180.

American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders*.

Auger, A.P. & Olesen, K.M., 2009. Brain sex differences and the organisation of juvenile social play behaviour. *Journal of Neuroendocrinology*, 21(6), pp.519–525.

Aultman, B., 2014. Cisgender. *TSQ: Transgender Studies Quarterly*, 1(1–2), pp.59–61.

Baldinger, P. et al., 2014. Impact of COMT genotype on serotonin-1A receptor binding investigated with PET. *Brain Structure and Function*, 219(6), pp.2017–2028.

Bandelow, B. et al., 2016. Biological markers for anxiety disorders, OCD and PTSD – a consensus statement. Part I: Neuroimaging and genetics. World Journal of Biological Psychiatry, 17(5), pp.321–365.

Bao, A.M. & Swaab, D.F., 2011. Sexual differentiation of the human brain: Relation to gender identity, sexual orientation and neuropsychiatric disorders. *Frontiers in Neuroendocrinology*, 32(2), pp.214–226.

Barnes, N.M. & Sharp, T., 1999. A review of central 5-HT receptors and their function. *Neuropharmacology*, 38(8), pp.1083–1152.

Barth, C., Villringer, A. & Sacher, J., 2015. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Frontiers in Neuroscience*, 9(FEB).

Basow, S. A. (1992). Gender: Stereotypes and roles (3rd ed.). Belmont, CA, US: Thomson Brooks/Cole Publishing Co.

Bear, M., Connors, B. & Paradiso, M., 2007. *Neuroscience: Eploring the brain*.

Benninghoff, A. 1985. Makroskopische und mikroskopische Anatomie des Menschen; Bd. 3., Nervensystem, Haut und Sinnesorgane. München ; Wien ; Baltimore : Urban und Schwarzenberg. XVI, 622 S. : 686 Ill

Bentz, E.K. et al., 2007. A common polymorphism of the SRD5A2 gene and transsexualism. *Reproductive Sciences*, 14(7), pp.705–709.

Bentz, E.K. et al., 2008. A polymorphism of the CYP17 gene related to sex steroid metabolism is associated with female-to-male but not male-to-female transsexualism. *Fertility and Sterility*, 90(1), pp.56–59.

Bettencourt, B.A. & Miller, N., 1996. Gender differences in aggression as a function of provocation: A meta-analysis. *Psychological Bulletin*, 119(3), pp.422–447.

Bharucha, a E. et al., 2000. Effects of a serotonin 5-HT(4) receptor antagonist SB-207266 on gastrointestinal motor and sensory function in humans. *Gut*, 47(5), pp.667–674.

Binder, J.R. et al., 1997. Human brain language areas identified by functional magnetic resonance imaging. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 17(1), pp.353–362.

Bioethikkommission, S. Der & Bioethikkommission, B.Ö., Intersexualität und Intersexualität und Transidentität Transidentität.

Bleich, H.L. et al., 1982. The Evolutionary Origins of Hormones, Neurotransmitters, and Other Extracellular Chemical Messengers. *New England Journal of Medicine*, 306(9), pp.523–527.

Bleier, R., Byne, W. & Siggelkow, I., 1982. Cytoarchitectonic sexual dimorphisms of the medial preoptic and anterior hypothalamic areas in guinea pig, rat, hamster, and mouse. *Journal of Comparative Neurology*, 212(2), pp.118–130.

Bobb, A.J. et al., 2005. Support for association between ADHD and two candidate genes: NET1 and DRD1. *American Journal of Medical Genetics - Neuropsychiatric Genetics*, 134 B(1), pp.67–72.

Bock, C., 2012. Analysing and interpreting DNA methylation data. *Nature reviews. Genetics*, 13(10), pp.705–719.

Bonnin, A. et al., 2007. Serotonin modulates the response of embryonic thalamocortical axons to netrin-1. *Nature Neuroscience*, 10(5), pp.588–597.

Bonnin, A. & Levitt, P., 2012. Placental source for 5-HT that tunes fetal brain development. *Neuropsychopharmacology*, 37(1), pp.299–300.

Brodmann, Korbinian, 1909. Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Barth.

Brookes, A.J., 2007. Single Nucleotide Polymorphism (SNP). *Life Sciences*, 14(5), pp.3–6.

Brookshire, B., 2015. Serotonin and the science of sex. *Science News*, 10 April, pp.5–7.

Brummelte, S. et al., 2017. Developmental changes in serotonin signaling: Implications for early brain function, behavior and adaptation. *Neuroscience*, 342, pp.212–231.

Buhrich, N., Barr, R. & Lam-Po-Tang, P.R.L.C., 1978. Two transsexuals with 47-XYY karyotype. *British Journal of Psychiatry*, 133(7), pp.77–81.

Canli, T. & Lesch, K.P., 2007. Long story short: The serotonin transporter in emotion regulation and social cognition. *Nature Neuroscience*, 10(9), pp.1103–1109.

Carlson, Bruce (15 June 2008). SNPs — A Shortcut to Personalized Medicine. Genetic Engineering & Biotechnology News. Mary Ann Liebert, Inc. 28 (12).

Cashdan, E., 2003. Hormones and Competitive Aggression in Women. *Aggressive Behavior*, 29(2), pp.107–115.

Caspi, A. et al., 2010. Genetic sensitivity to the environment:The case of the serotonin transporter gene and its implications for studying complex diseases and traits. American Journal of Psychiatry, 167(5), pp.509–527.

Caspi, A. et al., 2003. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*, 301(5631), pp.386–389.

Caspi, A. et al., 2002. Role of genotype in the cycle of violence in maltreated children. *Science*, 297(5582), pp.851–854.

Champagne, F.A., 2008. Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, 29(3), pp.386–397.

Chivers, M.L., 2017. The Specificity of Women's Sexual Response and Its Relationship with Sexual Orientations: A Review and Ten Hypotheses. *Archives of Sexual Behavior*, 46(5), pp.1161–1179.

Chou, Y. et al., 2013. Association of Brain Serotonin Transporter Availability and Brain-Derived Neurotrophic Factor in Models of Serotonin Transporter Genotypes in Healthy Subjects. journal of clinical psychopharmacology, 33(3), pp.432–435.

Chugani, D. et al., 1998. Human Brain Serotonin Synthesis Capacity Measured In Vivo with. Synapse (New York, N.Y.), 43(February 1997), pp.33–43.

Coolidge, F.L. et al., 2008. A polymorphism of the CYP17 gene related to sex steroid metabolism is associated with female-to-male but not male-to-female transsexualism. Fertility and Sterility, 32(4), pp.56–59.

„Corticalisation – Lexikon der Neurowissenschaft" 2018. Accessd August 18. https://www.spektrum.de/lexikon/neurowissenschaft/corticalisation/2457

Costa, P.T., Terracciano, A. & McCrae, R.R., 2001. Gender differences in personality traits across cultures: Robust and surprising findings. *Journal of Personality and Social Psychology*, 81(2), pp.322–331.

Craft, R.M., Mogil, J.S. & Maria Aloisi, A., 2004. Sex differences in pain and analgesia: The role of gonadal hormones. *European Journal of Pain*, 8(5), pp.397–411.

Curley, J.P., Mashoodh, R. & Champagne, F.A., 2011. Epigenetics and the origins of paternal effects. *Hormones and Behavior*, 59(3), pp.306–314.

Dankbar, B. et al., 1995. Quantification of androgen receptor and follicle-stimulating hormone receptor mRNA levels in human and monkey testes by a ribonuclease-protection assay. *International Journal of Andrology*, 18(2), pp.88–96.

Darwin, C. 1871. The descent of man, and selection in relation to sex. London: John Murray.

Davies, P.J., 2010. *Plant Hormones*, Springer Netherlands

Dekaban, A.S. & Sadowsky, D., 1978. Changes in brain weights during the span of human life: Relation of brain weights to body heights and body weights. *Annals of Neurology*, 4(4), pp.345–356.

DeLacoste-Utamsing, C. & Holloway, R.L., 1982. Sexual dimorphism in the human corpus callosum. *Science (New York, N.Y.)*, 216(4553), pp.1431–2.

Dewing, P. et al., 2006. Direct regulation of adult brain function by the male-specific factor SRY. *Current biology : CB*, 16(4), pp.415–20.

Döhler, K.D., 1991. The Pre- and Postnatal Influence of Hormones and Neurotransmitters on Sexual Differentiation of the Mammalian Hypothalamus. *International Review of Cytology*, 131(C), pp.1–57.

Dudel, J., Menzel, R. & Schmidt, R.F., 2002. *Neurowissenschaft - Vom Molekül zur Kognition*,

Ehlert, U., 2011. Das endorkine System. In *Psychoendokrinologie und Psychoimmunologie*. pp. 3–36.

Ehrhardt, A.A. & Meyer-Bahlburg, H.F.L., 1981. Effects of prenatal sex hormones on gender-related behavior. *Science*, 211(4488), pp.1312–1318.

Ellegren, H. & Parsch, J., 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics*, 8(9), pp.689–698.

Ellis, H. 1934. Man and woman. A study of secondary and tertiary sexual characters. Eighth Edition, revised. London, William Heinemann (Medical Books) LTD.

Etgen, A.M. & Morales, J.C., 2002. Somatosensory stimuli evoke norepinephrine release in the anterior ventromedial hypothalamus of sexually receptive female rats. *Journal of Neuroendocrinology*, 14(3), pp.213–218.

Fausto-Sterling, A., 1993. The five sexes: Why Male and Female are not Enough. *The Sciences*, 2(March/April), pp.20–24.

Fowler, J.S. et al., 1999. PET and Drug Research and Development *. *J. Nucl. Med.*, 40(7), pp.1154–1163.

Galea, S., Nandi, A. & Vlahov, D., 2005. The epidemiology of post-traumatic stress disorder after disasters. *Epidemiologic Reviews*, 27, pp.78–91.

Gandhi, S.P., Heeger, D.J. & Boynton, G.M., 1999. Spatial attention affects brain activity in human primary visual cortex. *Proceedings of the National Academy of Sciences of the United States of America*, 96(6), pp.3314–9.

Gaspar, P., Cases, O. & Maroteaux, L., 2003. The developmental role of serotonin: News from mouse molecular genetics. *Nature Reviews Neuroscience*, 4(12), pp.1002–1012.

Gaunt, T.R. et al., 2016. Systematic identification of genetic influences on methylation across the human life course. Genome Biology, pp.1–14.

Geary, D.C., 1998. *Male, female: The evolution of human sex differences.*

Ginovart, N. et al., 2006. Positron emission tomography quantification of [11C]-harmine binding to monoamine oxidase-A in the human brain. *Journal of Cerebral Blood Flow and Metabolism*, 26(3), pp.330–344.

Gizewski, E.R. et al., 2009. Specific cerebral activation due to visual erotic stimuli in male-to-female transsexuals compared with male and female controls: an fMRI study. *The Journal of Sexual Medicine*, 6(2), pp.440–448.

Gjedde, A., Bauer, W.R. & Wong, D.F., 2011. *Neurokinetics: The dynamics of neurobiology in vivo*,

Glenn, A.L., 2011. The other allele: Exploring the long allele of the serotonin transporter gene as a potential risk factor for psychopathy: A review of the parallels in findings. *Neuroscience and Biobehavioral Reviews*, 35(3), pp.612–620.

Gorski, R.A. et al., 1980. Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *The Journal of comparative neurology*, 193(2), pp.529–39.

Groenewegen, H.J., 2003. The basal ganglia and motor control. *Neural plasticity*, 10(1–2), pp.107–120.

Grossman, M. & Wood, W., 1993. Sex differences in intensity of emotional experience: a social role interpretation. *Journal of Personality and Social Psychology*, 65(5), pp.1010–1022.

Gray, J.1992. Men Are from Mars, Women Are from Venus. *Goodreads.*

Gryglewski, G. et al., 2014. Meta-analysis of molecular imaging of serotonin transporters in major depression. *Journal of Cerebral Blood Flow and Metabolism*, 34(7), pp.1096–1103.

Gudelsky, G.A., Koenig, J.I. & Meltzer, H.Y., 1986. Thermoregulatory responses to serotonin (5-ht) receptor stimulation in the rat. *Neuropharmacology*, 25(12), pp.1307–1313.

Guillamon, A., Junque, C. & Gomez-Gil, E., 2016. A Review of the Status of Brain Structure Research in Transsexualism. *Archives of Sexual Behavior*, 45(7), pp.1615–1648.

Haberman, M. et al., 1975. Gender identity confusion, schizophrenia and a 47 XYY karyotype: a case report. *Psychoneuroendocrinology*, 1(2), pp.207–209.

Hahn, A. et al., 2010. Escitalopram Enhances the Association of Serotonin-1A Autoreceptors to Heteroreceptors in Anxiety Disorders. *Journal of Neuroscience*, 30(43), pp.14482–14489.

Hahn, A. et al., 2015. Structural connectivity networks of transgender people. *Cerebral Cortex*, 25(10), pp.3527–3534.

Halpern, D.F. et al., 2007. The Science of Sex Differences in Science and Mathematics. *Psychological Science in the Public Interest: A Journal of the American Psychological Society*, 8(1), pp.1–51.

Hare, L. et al., 2009. Androgen Receptor Repeat Length Polymorphism Associated with Male-to-Female Transsexualism. *Biological Psychiatry*, 65(1), pp.93–96.

Hariri, A.R. et al., 2002. Serotonin transporter genetic variation and the response of the human amygdala. *Science (New York, N.Y.)*, 297(5580), pp.400–3.

Hausen, K. 2014. Die Polarisierung der Geschlechtscharaktere. Gender, Soziologie. Eine Spiegelung der Dissoziation von Erwerbs- und Familienleben", aus: Werner Conze (Hg.), Sozialgeschichte der Familie in der Neuzeit Europas. Neue Forschungen, Stuttgart 1976, Seiten 363-393

Heath, M.J.S. & Hen, R., 1995. Serotonin Receptors: Genetic insights into serotonin function. *Current Biology*, 5(9), pp.997–999.

Heils, A. et al., 1996. Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry*, 66(6), pp.2621–2624.

Henningsson, S. et al., 2005. Sex steroid-related genes and male-to-female transsexualism. *Psychoneuroendocrinology*, 30(7), pp.657–664.

Hines, M., 1982. Prenatal gonadal hormones and sex differences in human behavior. *Psychological Bulletin*, 92(1), pp.56–80.

Hirnstein, M., Coloma Andrews, L. & Hausmann, M., 2014. Gender-Stereotyping and Cognitive Sex Differences in Mixed- and Same-Sex Groups. *Archives of Sexual Behavior*, 43(8), pp.1663–1673.

Horn, Fl., 2009. Biochemie des Menschen: Das Lehrbuch für das Medizinstudium. *Thieme*, pp.330–425.

Hyde, C. & Kenna, J.C., 1977. A male M Z twin pair, concordant for transsexualism, discordant for schizophrenia. *Acta Psychiatrica Scandinavica*, 56(4), pp.265–273.

Hyde, J.S., 2005. The gender similarities hypothesis. *American Psychologist*, 60(6), pp.581–592.

Van Ijzendoorn, M.H. et al., 2010. Methylation matters: Interaction between methylation density and serotonin transporter genotype predicts unresolved loss or trauma. *Biological Psychiatry*, 68(5), pp.405–407.

Ichise, M., Liow, J.-S., Lu, J.-Q., Takano, A., Model, K., Toyama, H., Suhara, T., Suzuki, K., Innis, R.B., Carson, R.E., 2003. Linearized Reference Tissue Parametric Imaging Methods Application to 11C-DASB Positron Emission Tomography Studies of the Serotonin Transporter in Human Brain. J Cereb Blood Flow Metab 23, 1096-1112.

Ingalhalikar, M. et al., 2014. Sex differences in the structural connectome of the human brain. *Proceedings of the National Academy of Sciences of the United States of America*, 111(2), pp.823–828.

James, G.M. et al., 2017. Effects of Selective Serotonin Reuptake Inhibitors on Interregional Relation of Serotonin Transporter Availability in Major Depression. *Frontiers in Human Neuroscience*, 11.

Jeltsch, A., 2002. Beyond Watson and Crick: DNA Methylation and Molecular Enzymology of DNA Methyltransferases. *ChemBioChem*, 3(4), pp.274–293.

Jenuwein, T., 2006. The epigenetic magic of histone lysine methylation. *The FEBS journal*, 273(14), pp.3121–35.

Johnson, R.L. & Koerner, J.F., 1988. Excitatory Amino Acid Neurotransmission. *Journal of Medicinal Chemistry*, 31(11), pp.2057–2066.

Jones, P.A. & Takai, D., 2001. The role of DNA methylation in mammalian epigenetics. Science.

Jovanovic, H. et al., 2008. Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. , 39, pp.1408–1419.

Kang, H.J. et al., 2013. Association of SLC6A4 methylation with early adversity, characteristics and outcomes in depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 44, pp.23–28.

Kautzky, A. et al., 2017. The influence of the rs6295 gene polymorphism on serotonin-1A receptor distribution investigated with PET in patients with major depression applying machine learning. *Translational psychiatry*, 7(6), p.e1150.

Kim, D.Y. & Camilleri, M., 2000. Serotonin: a mediator of the brain-gut connection. *The American journal of gastroenterology*, 95(10), pp.2698–2709.

Kleine, B. & Rossmanith, W.G., 2016. Hormones: Definitions. In *Hormones and the Endocrine System: Textbook of Endocrinology*. pp. 16–7.

Konkle, A.T.M. & McCarthy, M.M., 2011. Developmental time course of estradiol, testosterone, and dihydrotestosterone levels in discrete regions of male and female rat brain. *Endocrinology*, 152(1), pp.223–235.

Kónya, J. & Nagy, N.M., 2012. *Nuclear and Radiochemistry*,

Kranz, GS., et al., 2014. Cerebral serotonin transporter asymmetry in females, males and male-to-female transsexuals measured by PET in vivo. *Brain Structure and Function* 219(1): 171-183.

Kranz, G.S. et al., 2013. Alterations in serotonin transporter asymmeregional cerebral try in male-to-female transsexuals measured by brain pet. *European Psychiatry*, 28, p.1.

Kranz, G.S. et al., 2017. Effects of sex hormone treatment on white matter microstructure in individuals with gender dysphoria. *NeuroImage*, 150(February), pp.60–67.

Kranz, G.S. et al., 2015. High-dose testosterone treatment increases serotonin transporter binding in transgender people. *Biological Psychiatry*, 78(8), pp.525–533.

Kranz, G.S. et al., 2014. White Matter Microstructure in Transsexuals and Controls Investigated by Diffusion Tensor Imaging. *Journal of Neuroscience*, 34(46), pp.15466–15475.

Kraus, C. et al., 2014. Exploring the impact of BDNF Val66Met genotype on serotonin transporter and serotonin-1A receptor binding. *PLoS ONE*, 9(9), pp.1–8.

Kring, A.M. & Gordon, A.H., 1998. Sex differences in emotion: expression, experience, and physiology. *Journal of Personality and Social Psychology*, 74(3), pp.686–703.

Ku, H.-L. et al., 2013. Brain signature characterizing the body-brain-mind axis of transsexuals. *PloS One*, 8(7), p.e70808.

Kunugi, H. et al., 1997. Serotonin transporter gene polymorphisms : ethnic difference and possible association with bipolar affective disorder. Molecular Psychiatry, 2, pp.457–462.

Kuyper, L. & Wijsen, C., 2014. Gender identities and gender dysphoria in the Netherlands. *Archives of Sexual Behavior*, 43(2), pp.377–385.

Langer, S.Z. et al., 1981. High-affinity binding of 3H-imipramine in brain and platelets and its relevance to the biochemistry of affective disorders. *Life Sciences*, 29(3), pp.211–220.

Lanzenberger, R. et al., 2010. Cortisol plasma levels in social anxiety disorder patients correlate with serotonin-1A receptor binding in limbic brain regions. *International Journal of Neuropsychopharmacology*, 13(9), pp.1129–1143.

Lanzenberger, R. et al., 2007. Reduced Serotonin-1A Receptor Binding in Social Anxiety Disorder. *Biological Psychiatry*, 61(9), pp.1081–1089.

Lanzenberger et. al 2009. Serotonin1A receptor binding and Reward-dependent Activation are associated within the Human Dorsal Raphe Nucleus as revealed by PET-fMRI. *NeuroImage, 47(1), p.176.*

Lesch, K., 2007. Linking emotion to the social brain. *EMBO Reports*, 8(10), pp.S24–S29.

Lesch, K.P. et al., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*, 274(5292), pp.1527–1531.

Levy, J. & Heller, W., 1992. Gender differences in human neuropsychological function. In *Sexual differentiation*. pp. 245–274.

Lindsey, Linda L. (2015). "The sociology of gender" Gender roles: a sociological perspective. Boston: Pearson. p. 4. ISBN 9780205899685.

Liu, Y. et al., 2011. Molecular regulation of sexual preference revealed by genetic studies of 5-HT in the brains of male mice. *Nature*, 472(7341), pp.95–100.

Lodish, H., Berk, A. & Zipursky SL, et al., 2000. Neurotransmitters, Synapses, and Impulse Transmission. In *Molecular Cell Biology. 4th edition*. pp. 46–7.

Löffler, G., 2008. *Basiswissen Biochemie*. Springer-Lehrbuch

Luders, E. et al., 2006. Gender effects on cortical thickness and the influence of scaling. *Human Brain Mapping*, 27(4), pp.314–324.

Luders, E. et al., 2012. Increased Cortical Thickness in Male-to-Female Transsexualism. *Journal of behavioral and brain science*, 2(3), pp.357–362.

Luders, E. & Toga, A.W., 2010. Chapter 1 - Sex differences in brain anatomy. *Progress in Brain Research*, 186, pp.2–12.

MacLean, H.E. et al., 1993. Related individuals with different androgen receptor gene deletions. *The Journal of clinical investigation*, 91(3), pp.1123–8.

Manuck, S.B. et al., 2000. A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. *Psychiatry Research*, 95(1), pp.9–23.

Manzouri, A., Kosidou, K. & Savic, I., 2017. Anatomical and Functional Findings in Female-to-Male Transsexuals: Testing a New Hypothesis. *Cerebral cortex (New York, N.Y. : 1991)*, 27(2), pp.998–1010.

Martinowich, K. & Lu, B., 2008. Interaction between BDNF and serotonin: Role in mood disorders. *Neuropsychopharmacology*, 33(1), pp.73–83.

Matsumoto, A. & Arai, Y., 2008. Male-Female Difference in Synaptic Organization of the Ventromedial Nucleus of the Hypothalamus in the Rat. *Neuroendocrinology*, 42(3), pp.232–236.

McCarthy, M.M. et al., 2009. The epigenetics of sex differences in the brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(41), pp.12815–23.

McCarthy, M.M. & Nugent, B.M., 2015. At the frontier of epigenetics of brain sex differences. *Frontiers in Behavioral Neuroscience*, 9(August), pp.1–8.

McCarthy, M.M., 2016. Multifaceted origins of sex differences in the brain. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1688).

Mcqueen, J.K. et al., 1999. Serotonin transporter ž SERT / mRNA and binding site densities in male rat brain affected by sex steroids. , pp.241–247.

Meguid, M.M. et al., 2000. Hypothalamic dopamine and serotonin in the regulation of food intake. In *Nutrition*. pp. 843–857.

Meyer, J.H. et al., 2004. Brain Serotonin Transporter Binding Potential Measured With Carbon 11–Labeled DASB Positron Emission Tomography. *Arch Gen Psychiatry*, 61(12), pp.1271–1279.

Meyer zu Hoberge, S., 2009. Prävalenz, Inzidenz Und Geschlechterverhältnis Der Transsexualität Anhand Der Bundesweit Getroffenen Entscheidungen Nach Dem Transsexuellengesetz in Der Zeit Von 1991 Bis 2000.

Millan, M.J. et al., 2008. Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. *Trends in pharmacological sciences*, 29(9), pp.454–64.

Miller, A.K.H. & Corsellis, J.A.N., 1977. Evidence for a secular increase in human brain weight during the past century. *Annals of Human Biology*, 4(3), pp.253–257.

Mintun, M.A. et al., 1984. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Annals of Neurology*, 15(3), pp.217–227.

Mitsushima, D. et al., 2006. Sex differences in the basolateral amygdala: the extracellular levels of serotonin and dopamine, and their responses to restraint stress in rats. *The European journal of neuroscience*, 24(11), pp.3245–54.

Moore, L.D., Le, T. & Fan, G., 2013. DNA methylation and its basic function. *Neuropsychopharmacology*, 38(1), pp.23–38.

Morris, J.A., Jordan, C.L. & Breedlove, S.M., 2004. Sexual differentiation of the vertebrate nervous system. *Nature Neuroscience*, 7(10), pp.1034–1039.

Ngun, T.C. et al., 2011. The genetics of sex differences in brain and behavior. *Frontiers in Neuroendocrinology*, 32(2), pp.227–246.

Nicoletti, R., Oberladstätter, M. & König, F., 2007. *Messtechnik und Instrumentierung in der Nuklearmedizin* 2th ed., facultas.

Nishizawa, S. et al., 1997. Differences between males and females in rates of serotonin synthesis in human brain. *Proceedings of the National Academy of Sciences of the United States of America*, 94(10), pp.5308–5313.

Oeth P, Beaulieu M, Park C, Kosman D, del Mistro G, van den Boom D, Jurinke C (2007) iPLEXTM assay: increased plexing efficiency and flexibility for MassARRAYÒ system through single base primer extension with mass-modified terminators. SEQUENOMÒ Application Note.

Offermanns, S., 2012. Hypothalamus-Hypophysen-System. In *Pharmakologie und Toxikologie: Von den molekularen Grundlagen zur Pharmakotherapie*. p. 524 526.

Okada, S. et al., 2014. The potential of SLC6A4 gene methylation analysis for the diagnosis and treatment of major depression. *Journal of Psychiatric Research*, 53(1), pp.47–53.

Von Der Pahlen, B. et al., 2002. An Exploratory Study on Self-Evaluated Aggression and Androgens in Women. *Aggressive Behavior*, 28(4), pp.273–280.

Parsey, R. V et al., 2006. Lower serotonin transporter binding potential in the human brain during major depressive episodes. The American journal of psychiatry, 163(1), pp.52–8.

Pate, J., 2012. What everyone should know about gender and sexuality. jamespatemd.com. Archived on 17 July 2018.

Philibert, R. et al., 2007. Serotonin transporter mRNA levels are associated with the methylation of an upstream CpG island. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, 144(1), pp.101–105.

Philibert, R.A. et al., 2008. Rapid publication: The relationship of 5HTT (SLC6A4) methylation and genotype on mRNA expression and liability to major depression and alcohol dependence in subjects from the Iowa adoption studies. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, 147(5), pp.543–549.

Phoenix, C.H. et al., 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*, 65, pp.369–382.

Pickering, G. et al., 2003. Serotonin and experimental pain in healthy young volunteers. *The Clinical journal of pain*, 19(4), pp.276–279.

Polderman, T.J.C. et al., 2018. The Biological Contributions to Gender Identity and Gender Diversity: Bringing Data to the Table. *Behavior Genetics*, 48(2), pp.95–108.

Ponseti, J. et al., 2006. A functional endophenotype for sexual orientation in humans. *NeuroImage*, 33(3), pp.825–833.

Praschak-Rieder, N. et al., 2008. Seasonal variation in human brain serotonin transporter binding. *Arch Gen Psychiatry*, 65(9), pp.1072–1078.

Purves, D. et al., 2004. *Neuroscience*

Raisman, R., Briley, M. & Langer, S.Z., 1979. Specific tricyclic antidepressant binding sites in rat brain. *Nature*, 281(5727), pp.148–150.

Ramamoorthy, S. et al., 2016. Phosphorylation and Sequestration of Serotonin Transporters Differentially Modulated by Psychostimulants. American Association for the Advancement of Science, 285(5428), pp.763–766.

Reed, B.G. & Carr, B.R., 2015. *The Normal Menstrual Cycle and the Control of Ovulation*,

Reimold, M. et al., 2007. Midbrain serotonin transporter binding potential measured with [11C]DASB is affected by serotonin transporter genotype. *Journal of Neural Transmission*, 114(5), pp.635–639.

Rhees, R.W. et al., 1999. Relationship between sexual behavior and sexually dimorphic structures in the anterior hypothalamus in control and prenatally stressed male rats. *Brain Research Bulletin*, 50(3), pp.193–199.

Rizzolatti, G., Fogassi, L. & Gallese, V., 1997. Parietal cortex: From sight to action. *Current Opinion in Neurobiology*, 7(4), pp.562–567.

Ruigrok, A.N. V. et al., 2014. A meta-analysis of sex differences in human brain structure. *Neuroscience and Biobehavioral Reviews*, 39, pp.34–50.

Sacher, G.A. & Staffeldt, E.F., 1974. Relation of Gestation Time to Brain Weight for Placental Mammals: Implications for the Theory of Vertebrate Growth. *The American Naturalist*, 108(963), pp.593–615.

Sadeghi, M. & Fakhrai, A., 2000. Transsexualism in female monozygotic twins: A case report. *Australian and New Zealand Journal of Psychiatry*, 34(5), pp.862–864.

Safron, A. et al., 2018. Neural Correlates of Sexual Orientation in Heterosexual, Bisexual, and Homosexual Women. *Scientific Reports*, 8(1).

Schicha, H. & Schober, O., 2013. *Nuklearmedizin - Basiswissen und klinische Anwendung* 7th ed., Stuttgart, Germany: Schattauer.

Schneider, I. et al., 2017. Association of Serotonin Transporter Gene AluJb Methylation with Major Depression, Amygdala Responsiveness, 5-HTTLPR/rs25531 Polymorphism, and Stress. *Neuropsychopharmacology*, (October).

Schoenwolf, G.C. & Smith, J.L., 1990. Mechanisms of neurulation: traditional viewpoint and recent advances. *Development (Cambridge, England)*, 109(2), pp.243–270.

Schön, H., Ordnung, W. & Schmid, E., 1960. Zum Nachweis der 5-Hydroxyind Olessigsäure im Harn. In *Molecular Medicine*. pp. 405–406.

Schonfeld WA, 1943. primary and secondary sexual characteristics. *American Journal of disease in childhood*, 65, p.353.

Schuebel, K. et al., 2016. Making sense of epigenetics. *International Journal of Neuropsychopharmacology*, 19(11), pp.1–10.

Schwaiger, M. & Pirich, C., 2000. Positron emission tomography. *Positronen-emissions-tomographie*, 89(SUPPL. 1), pp.59–66.

Segal, N.L., 2006. Two monozygotic twin pairs discordant for female-to-male transsexualism. *Archives of Sexual Behavior*, 35(3), pp.347–358.

Selye, H., 1941. Pharmacological classification of steroid hormones [3]. *Nature*, 148(3742), pp.84–85.

Sharp, T. & Hjorth, S., 1990. Application of brain microdialysis to study the pharmacology of the 5-HT1A autoreceptor. *Journal of Neuroscience Methods*, 34(1–3), pp.83–90.

Sigurdardottir, H.L. et al., 2016. Effects of norepinephrine transporter gene variants on NET binding in ADHD and healthy controls investigated by PET. *Human Brain Mapping*, 37(3), pp.884–895.

Sigusch, V., 2005. *Neosexualitäten: Über den kulturellen Wandel von Liebe und Perversion*.

Siiteri, P.K., 1979. Sex hormone production and action. *Arthritis & Rheumatism*, 22(11), pp.1284–1294.

Snaith, R.P., Penhale, S. & Horsfield, P., 1991. Male-to-female transsexual with XYY karyotype. *The Lancet*, 337(8740), pp.557–558.

Sodhi, M.S.K. & Sanders-Bush, E., 2003. Serotonin and brain development. *International Review of Neurobiology*, 59, pp.111–174.

Spies, M. et al., 2015. The serotonin transporter in psychiatric disorders: Insights from PET imaging. *The Lancet Psychiatry*, 2(8), pp.743–755.

Spies, M. et al., 2016. Gender transition affects neural correlates of empathy: A resting state functional connectivity stud. NeuroImage, 5(138), pp.257–265.

Staley, J.K. et al., 2001. Sex differences in [123I]beta-CIT SPECT measures of dopamine and serotonin transporter availability in healthy smokers and nonsmokers. *Synapse (New York, N.Y.)*, 41(4), pp.275–284.

Suchiman HE, Slieker RC, Kremer D, Slagboom PE, Heijmans BT, Tobi EW, 2015. Design, measurement and processing of region-specific DNA methylation assays: the mass spectrometry-based method EpiTYPER. *Front Genet*; 6: 287.

Swaab, D.. & Garcia-Falgueras, A., 2009. Sexual differentiation of the human brain in relation to gender identity and sexual orientation. *Functional Neurology*, 24(No. 1), pp.17–28.

Swaab, D.F., 2007. Sexual differentiation of the brain and behavior. *Best Practice & Research. Clinical Endocrinology & Metabolism*, 21(3), pp.431–444.

Swaab, D.F., 2004. Sexual differentiation of the human brain: Relevance for gender identity, transsexualism and sexual orientation. *Gynecological Endocrinology*, 19(6), pp.301–312.

Swaab, D. & Bao, A.-M., 2013. Sexual differentiation of the human brain in relation to gender-identity, sexual orientation, and neuropsychiatric disorders.

Szalkai, B., Varga, B. & Grolmusz, V., 2015. Graph theoretical analysis reveals: Women's brains are better connected than men's. *PLoS ONE*, 10(7).

Tannen, D., 1991. *You just don't understand*, New York: Ballantine Books.

Thomas, P.E. et al., 2011. Challenges in the association of human single nucleotide polymorphism mentions with unique database identifiers. *BMC Bioinformatics*, 12(SUPPL. 4).

Trivers, R.L.L., 1972. Parental investment and sexual selection. *Sexual selection and the descent of man*, pp.136–179.

van Trotsenburg, M., 2018. Genderinkongruenz, das Gehirn und die Hormone. *Journal für Klinische Endokrinologie und Stoffwechsel*, 11(1), pp.12–18.

Tsukahara, S., 2009. Sex differences and the roles of sex steroids in apoptosis of sexually dimorphic nuclei of the preoptic area in postnatal rats. In *Journal of Neuroendocrinology*. pp. 370–376.

Tuominen, L. et al., 2017. Neuroticism associates with cerebral in vivo serotonin transporter binding differently in males and females. *International Journal of Neuropsychopharmacology*, 20(12), pp.963–970.

Turkheimer, F., Mattia, V. & Dunn, J., 2014. *Experimental Design and Practical Data Analysis in Positron Emission Tomography*,

Tyce, G.M., 1990. Origin and metabolism of serotonin. *Journal of Cardiovascular Pharmacology*, 16, pp.S1–S7.

Tzourio-Mazoyer, N. et al., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, 15(1), pp.273–289.

Ursin, R., 2002. Serotonin and sleep. *Sleep Medicine Reviews*, 6(1), pp.55–67.

Vijayendran, M. et al., 2012. Effects of genotype and child abuse on DNA methylation and gene expression at the serotonin transporter. *Frontiers in psychiatry / Frontiers Research Foundation*, 3(June), p.55.

Walker, C.L. & Ho, S.M., 2012. Developmental reprogramming of cancer susceptibility. Nature Reviews Cancer, 12(7), pp.479–486.

Wang, D. et al., 2012. Peripheral SLC6A4 DNA methylation is associated with in vivo measures of human brain serotonin synthesis and childhood physical aggression. PLoS ONE, 7(6), pp.3–10.

Wankerl, M. et al., 2014. Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles. *Translational Psychiatry*, 4.

Way, B.M. & Taylor, S.E., 2010. The serotonin transporter promoter polymorphism is associated with cortisol response to psychosocial stress. Biol Psychiatry, 67(5), pp.487–492.

Weiss, L.A. et al., 2005. Sex-Specific Genetic Architecture of Whole Blood Serotonin Levels. *The American Journal of Human Genetics*, 76(1), pp.33–41.

Whitaker-Azmitia, P.M., 2001. Serotonin and brain development: Role in human developmental diseases. In *Brain Research Bulletin*. pp. 479–485.

Whitaker-Azmitia, P.M. et al., 1995. Serotonin as a developmental signal. *Behavioural Brain Research*, 73(1–2), pp.19–29.

Wilkinson, M.B. et al., 2009. Imipramine Treatment and Resiliency Exhibit Similar Chromatin Regulation in the Mouse Nucleus Accumbens in Depression Models. *Journal of Neuroscience*, 29(24), pp.7820–7832.

Winter, S. et al., 2016. The Proposed ICD-11 Gender Incongruence of Childhood Diagnosis: A World Professional Association for Transgender Health Membership Survey. *Archives of Sexual Behavior*, 45(7), pp.1605–1614.

Wizemann, T.M. & Pardue, M., 2001. 3 Sex begins in the womb. In *Exploring the Biological Contributions to Human Health Does Sex Matter ?*. pp. 45–77.

Xu, K.P. et al., 1992. Sex-related differences in developmental rates of bovine embryos produced and cultured in vitro. *Molecular Reproduction and Development*, 31(4), pp.249–252.

Yildiz, O., Smith, J.R. & Purdy, R.E., 1998. Serotonin and vasoconstrictor synergism. *Life Sciences*, 62(19), pp.1723–1732.

Yoo, C., Ramirez, L. & Liuzzi, J., 2014. Big data analysis using modern statistical and machine learning methods in medicine. *International Neurourology Journal*, 18(2), pp.50–57.

Zhou, J.N. et al., 1995. A sex difference in the human brain and its relation to transsexuality. *Nature*, 378(6552), pp.68–70.

Zilles, K. & Rehkämper, G., 1993. *Funktionelle Neuroanatomie: Neuroendokrines System*, Springer-Verlag Berlin Heidelberg 1993.

Zimmer, L., 2009. Positron emission tomography neuroimaging for a better understanding of the biology of ADHD. *Neuropharmacology*, 57(7–8), pp.601–607.

Zubiaurre-Elorza, L. et al., 2013. Cortical thickness in untreated transsexuals. *Cerebral cortex (New York, N.Y. : 1991)*, 23(12), pp.2855–2862.

9. Appendix

9.1 Abbreviations

5-HT	serotonin (5-hydroxytryptamine)
5-HTT	serotonin transporter
5-HTTLPR	serotonin transporter promoter
ACC	anterior cingulate cortex
ADHD	Attention Deficit Hyperactivity Disorder
ALL	Automated Anatomical Labeling
BP	binding potential
BP_{ND}	nondisplaceable binding potential
CBG	corticosteroid binding globulin
CNS	central nervous system
CpG	cytosine-phosphate-guanine
CTh	cortical thickness
DASB	3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile
DNA	deoxyribonucleic acid
DRN	dorsal raphe nucleus
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	electrocardiography
EDTA	EthyleneDiamineTetraace- tic Acid
FSH	follicle-stimulating hormon
FtM	female-to-male
GABA	gamma-aminobutyric acid
GnRH	gonadotropin-releasing hormone
HPLC	high-performance liquid chromatography
HTR2A	serotonin receptor 2A
ICD	Internationale statistische Klassifikation der Krankheiten und verwandter Gesundheitsprobleme
L	long allele
LCWs	lobar connectivity weights
LH	luteinising hormone
MAO-A	monoamine oxidase A

MCC	medial cingulate cortex
MDD	Major Depressive Disorder
MDE	Major depressive episodes
MeCP2	methyl CpG binding protein 2
MRI	Magnetic Resonance Imaging
MRN	medial raphe nuclei
MRTM2	multilinear reference tissue model
MtF	male-to-female
PET	positron emission tomography
ROI	regions of interest
S	short allele
SCID	Structural Clinical Interview
SHBG	sex hormone binding proteins globulin
SLC6A4	serotonin transporter gene (Solute Carrier Family 6 Member 4)
SNP	single nucleotide polymorphism
SOC	Standards of Care
β^+	beta decay

9.2 Figures

Figure 1: Brain connections. Male brain is depicted on the left side, showing increased intra-hemispheric connections (blue). Female brain is depicted on the right brain and shows higher inter-hemispheric connections (orange). Taken and adapted from (Ingalhalikar et al. 2014)……..11

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