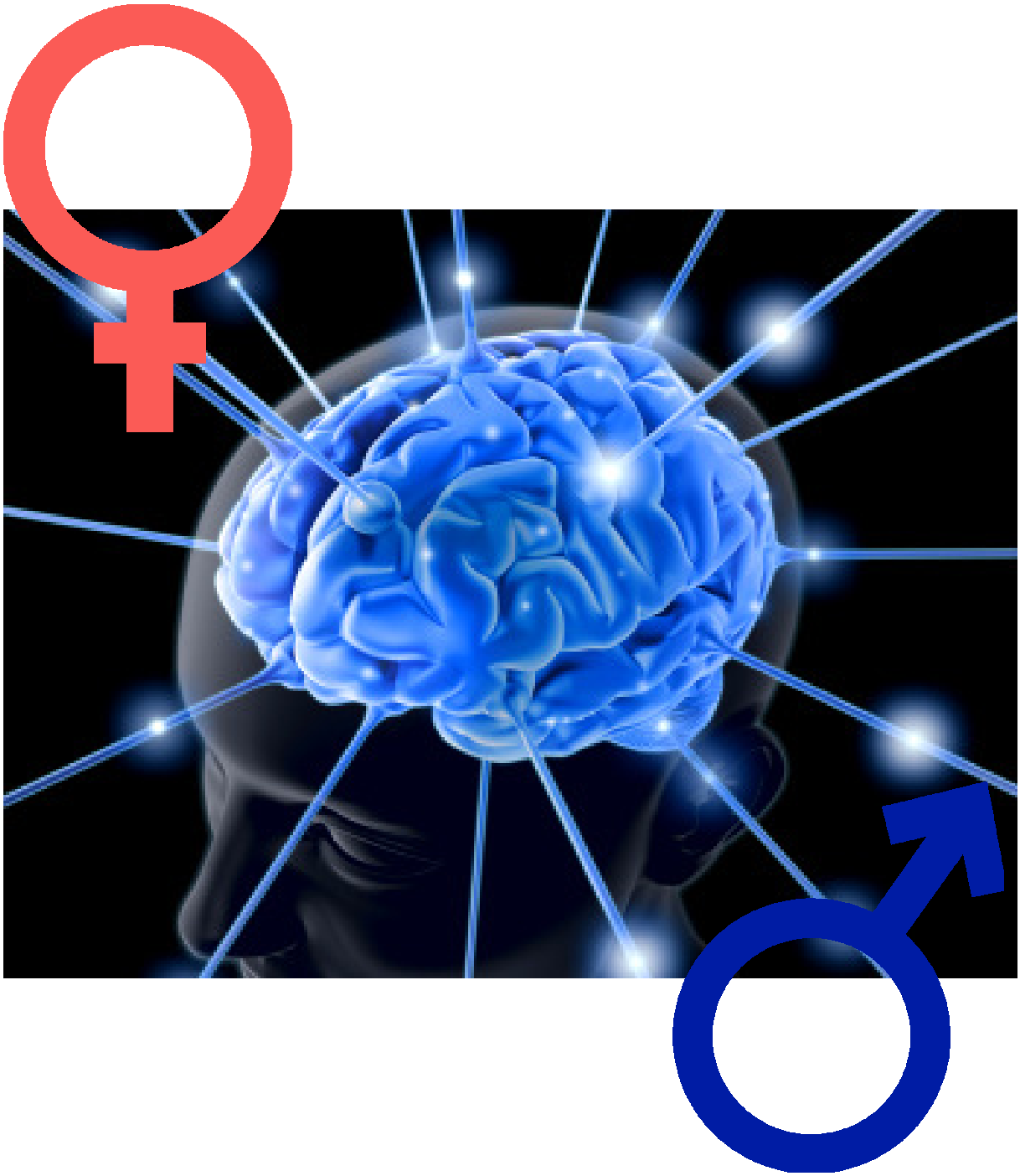
The Role of Sex Steroids in Sexual Differentiation of the Human Brian



Literature Thesis

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Contents

1. Abstract

Evidence shows that testosterone and estrogens play a major role in the sexual differentiation of the brain and the subsequent behavioral and cognitive differences between men and women. The brain is thought to develop in the male direction due to organizing effects of both testosterone and estrogens during prenatal development and increases in testosterone levels during early neonatal development and puberty. Female brain development is considered to occur due to the absence of sex steroid action during prenatal development and subsequent surges of estrogens during the neonatal period and puberty. Interactions between sex steroids and the brain during specific organizational periods in early life are hypothesized to form the basis of some core aspects of male and female differentiation, i.e. gender identity, sexual orientation and sexual behavior. Indeed, disorders of sex steroids, such as congenital adrenal hyperplasia and complete androgen insensitivity syndrome, show that distortion of sex steroid action early in human life permanently affects later behavior. Changes in sex steroid levels during puberty and their activational effects are closely related to sex differences as well. The higher testosterone levels in men compared to women are thought to result in sex differences in aggression, risk-taking and visuospatial abilities, with men showing higher levels of aggression and risk-taking and increased visuospatial performance. The higher estrogen levels, specifically the higher estradiol levels, in women compared to men are thought to be responsible for the better verbal episodic memory in women. However, many of the exact neurobiological mechanisms by which these sex steroids affect the described functions, remain to be determined by future research.

Keywords: brain differentiation, sex differences, testosterone, estradiol, behavior, cognition

2. Introduction

Besides the apparent physical differences, men and women exhibit differences in other domains, including (sexual) behavior and cognition (Bao & Swaab, 2010). Sex differences in behavior are present from birth onward. For instance, male newborns spend more time looking at mechanical mobiles while female newborns look more at human faces on their first day of life (Connelan et al., 2000). An apparent difference in childhood is playing behavior. Whereas boys prefer to play with toy cars and balls, girls devote most of their time to playing with dolls. This divergence is already present in infants of 3 to 8 months of age (Alexander et al., 2009). The observation that a similar differentiation in playing behavior is evident in non-human primates excludes the possibility that differences in playing behavior are the result of social pressure alone and points to possible biological underpinnings of these sex differences (Alexander & Hines, 2002).

Clear sexual dimorphism exists with respect to aggressive behavior as well. Men are more prone to show physical aggression. Men commit 89% of all murders and 99% of all sexual crimes (Spratt, 2000). Whereas women are more likely to show forms of indirect and verbal aggression (Hess & Hagen, 2006). Men and women do not only differ in behavior, but differences in cognitive performance are also present. Men for example have better visuospatial abilities (Martin et al., 2008), whereas women have a better episodic and verbal memory (Maki & Dumas, 2009). Sex differences are thus present in a wide variety of behavioral and cognitive domains. In addition, sexual differentiation is apparent in the prevalence of neuropsychiatric disorders. The proportions of cases range from more than 71% men in attention-deficit hyperactivity disorder, dyslexia, schizophrenia and autism to more than 67% female in anorexia, anxiety disorders and Alzheimer's disease (Swaab et al., 2003). The wide variation between men and women in the prevalence of neuropsychiatric disorders, in cognitive functions and in behavior strongly indicates possible biological differences.

Indeed, the numerous sex differences likely reflect differences in the brain between men and women. The male sex hormone testosterone and female estrogens are not only thought to be important for the sexual differentiation of the reproductive system but are considered to play a major role in the sexual differentiation of the brain as well. These sex steroids have been implicated in sexual differentiation because their levels differ between men and women and vary during development. Interactions between these sex steroids and the brain during development and adulthood are hypothesized to contribute significantly to sexual differentiation of the brain and to the subsequent differences in behavior and cognition (Bocklandt & Vilain, 2007).

Support for this assumption is provided by disorders of sex-steroids in humans, resulting in either too high or too low levels of sex steroids or their active receptors. One such a disorder is congenital adrenal hyperplasia (CAH). Girls with this disorder are exposed to high levels of

androgens, mainly testosterone, during prenatal development. These girls develop partially masculinized external genitalia and also show more male-typical behaviors even though they receive postnatal hormone treatment (Hines et al, 2004). CAH girls show more male-typical playing behavior and in addition, bi- and homosexuality and dissatisfaction with the female gender is more often reported in this group than in other females. The aberrant behavior in this patient group points to the importance of testosterone in (male) brain differentiation. Complete androgen insensitivity syndrome (CAIS) provides more support for an important contribution of sex steroids. These XY individuals have a mutation in the androgen receptor gene and are therefore resistant to the effects of androgens such as testosterone. Though these individuals are genetically male and have intra-abdominal testis, they develop a female phenotype due to the absence of androgen action. They show female-typical behavior and are satisfied with their female gender (Hines et al., 2003). Hence, both CAH and CAIS highlight the important role of the sex-steroids in male and female brain differentiation.

Evidence from these clinical studies provided some of the first support that sex-steroids are important in human sexual brain differentiation. In this thesis the current knowledge regarding the role of testosterone and estrogens in human sexual brain differentiation will be discussed. This thesis will not only discuss the effects of estrogens and testosterone on sex-related behavior but will also present the prominent non-reproductive influences of these sex steroids on the brain. The effects of testosterone and estrogens on the brain will be used to explain some of the well known differences in behavior, cognition and brain function between men and women.

3. Testosterone and Estrogens in the Reproductive System

In this chapter the effects of testosterone and estrogens related to reproduction and sexuality will be discussed. Testosterone and estrogens belong to the class of sex steroids and are also called sex hormones or gonadal hormones. In humans testosterone is the most important androgen and almost all research focuses on testosterone. Estrogens are more generally studied, though estradiol is the most important estrogen. Therefore this thesis will focus on the effects of testosterone and estrogens. Testosterone and estrogens exert their effects via androgen and estrogen receptors. The effects of sex steroids can be mediated through nuclear receptors leading to slow genomic effects or through membrane-associated receptors and signaling cascades leading to fast nongenomic mechanisms. Androgen and estrogen receptors are present in various tissues including the gonads and the brain, resulting in a wide variety of sex-steroid actions. Both estrogens and testosterone are synthesized via several enzymatic actions from the precursor cholesterol. The enzyme aromatase converts testosterone into estradiol. Estrogens and testosterone are most prominently produced by the gonads (testis in males, ovaries in

females) and in lesser amount by the adrenal gland and peripheral conversion of precursors. Testosterone and estrogens can be free in the blood plasma or bound to globulins, such as sex hormone binding globulin (SHBG), which makes these sex steroids inactive. The secretion of these sex steroids is regulated by a system involving the hypothalamus, pituitary and gonads, called the hypothalamic-pituitary-gonadal (HPG) axis (Piñón, 2002).

3.1. The HPG-axis

The central regulator of the HPG-axis and reproductive activity is the GnRH pulse generator, a group of neurons in the hypothalamus which secretes the peptide hormone gonadotropin releasing hormone (GnRH). GnRH (also called LHRH) is secreted in a pulsatile fashion, with a pulse frequency of one pulse every 60 minutes in humans. Via the hypophyseal portal system, GnRH reaches the pituitary where it stimulates cells of the anterior pituitary to the pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the blood stream. In females FSH and LH stimulate the ovaries to produce estrogen, progesterone and inhibin and regulate the menstrual cycle. In men LH and FSH act on the testes to regulate testosterone production and spermatogenesis respectively. The gonadal products testosterone, estrogen, progesterone and inhibin regulate the HPG-axis by negative feedback (Piñón, 2002).

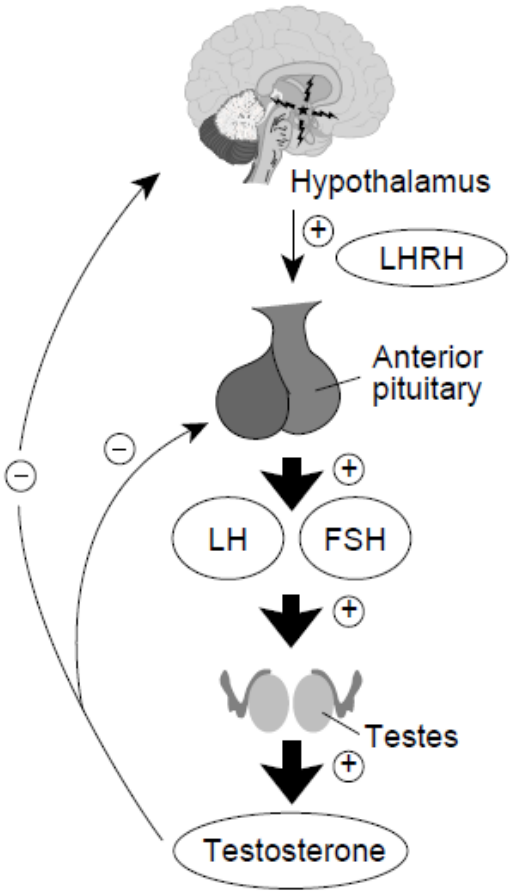

Figure 1. The male HPG-axis. GnRH, also called LHRH, is released by the hypothalamus and stimulates the anterior pituitary to secrete LH and FSH. LH and FSH stimulate the testes to produce testosterone and inhibin (not displayed). Testosterone and inhibin exert a negative feedback on the system.

3.1.1. The HPG-axis in Men

In men LH stimulates the Leydig cells in the testis to the production of testosterone. FSH on the other hand, interacts with receptors on the Sertoli cells in the testis, which results in spermatogenesis and the release of inhibin. Testosterone and inhibin regulate their own release by negative feedback (figure 1). Inhibin inhibits the release of LH and FSH by the pituitary, whereas testosterone inhibits both the release of LH and FSH by the pituitary and GnRH release by the hypothalamus. This results in a tight control of both testosterone release

and spermatogenesis. However, it is actually estradiol, which is synthesized from testosterone by aromatase, that is the direct inhibitor of the HPG-axis (Piñón, 2002).

3.1.2. The HPG-axis in Women

In women LH and FSH stimulate the ovaries to the production of progesterone, estrogen (including estradiol) and inhibin, which exert a negative feedback on GnRH and LH + FSH release by the hypothalamus and pituitary respectively (see figure 2). However, during puberty the regulation of the HPG-axis in women becomes much more complicated. In cycling women LH and FSH secretion varies drastically during the menstrual cycle. During the first two weeks of the menstrual cycle the LH pulse frequency increases and higher concentrations of LH are present in the blood compared to the luteal phase (last fourteen days of the cycle). Levels of LH increase even more as the LH surge approaches. The LH surge induces ovulation, the follicle is released and the luteal phase is initiated. The rapid increase in LH is due to the rapid rise in estrogen levels during the latter part of the follicular phase. Estrogen levels increase rapidly because their feedback to the hypothalamus and the pituitary changes from negative to positive just before the LH surge, though the exact mechanisms remain poorly understood. The positive feedback of estrogens is unique to females. After ovulation the GnRH pulse frequency decreases and levels of LH and FSH decrease as well. Levels of progesterone and estrogen increase again since they are produced by the corpus luteum, the remnant of the follicle. If fertilization does not occur, the corpus luteum regresses and estrogen and progresterone levels decline leading to menstruation in the last week of the luteal phase. FSH levels follow a similar pattern as LH levels, though during ovulation FSH levels are slightly lower. In postmenopausal women the situation changes due the relative absence of ovarian activity. A large increase in LH and FSH levels and their pulse frequency occurs. Due the relative low levels of estrogen

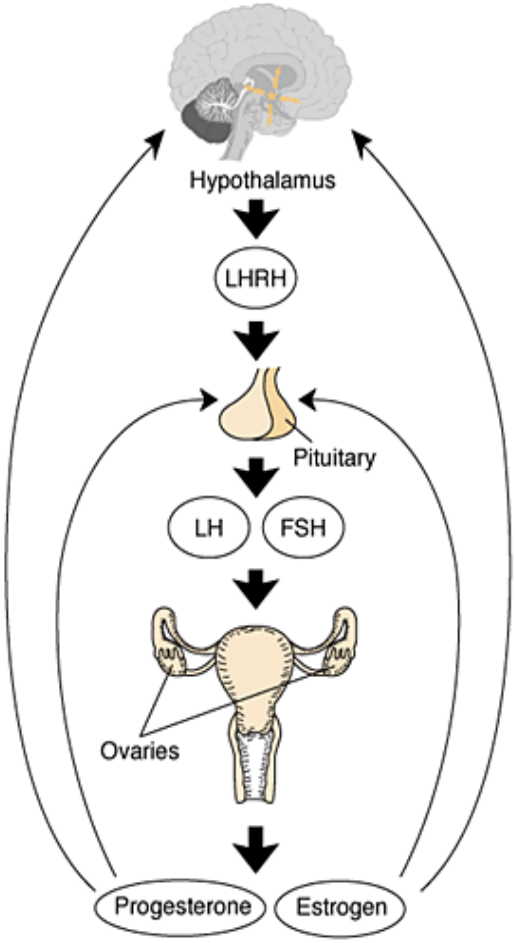

Figure 2. The female HPG-axis. GnRH, also called LHRH, is released by the hypothalamus and stimulates the anterior pituitary to secrete LH and FSH. LH and FSH stimulate the ovaries to produce progesterone, estrogen and inhibin (not displayed), which exert a negative feedback on the system.

and progesterone, negative feedback is lacking and LH and FSH levels remain high (Piñón, 2002).

3.2. Sex Steroids in the Development of the Male Reproductive System

For male development, three critical periods are present in which testosterone levels are relatively high. These three periods are mid-gestation during which the male sex-organs are formed, the first six months after birth, called minipuberty, and puberty during which the secondary sex characteristics develop and the body becomes capable of reproduction (Larsen et al., 2001).

Until the sixth week of fetal development the male and female genital systems are identically. In both the male and female fetus germ cells and sex cords are present in cortical and medullary regions of the presumptive gonads, accompanied by mesonephric and paramesonephric ducts. At the start of the seventh week this indifferent phase of genital development ends and the male and female systems pursue diverging pathways. The sex-determining region of the Y chromosome (SRY) is the transcription factor to initiate the cascade of events leading to male and female sexual dimorphism. When the fetus is genetically male (XY) and the transcription factor SRY is synthesized in the sex cord cells of the indifferent gonad, male development is initialized. In the absence of SRY, female development occurs. The SRY protein induces the cells of the medullary sex cords to differentiate into Sertoli cells and to secrete antimüllarian hormone (AMH) in the male fetus, while the cortical sex cord cells degenerate. AMH induces the degeneration of the paramesonephric (müllerian) ducts. In addition, in the ninth and tenth week, SRY signaling also induces the formation of Leydig cells which secrete testosterone. Between eight and ten weeks testosterone gives rise to the formation of the vas deferens and ductuli efferentes out of the mesonephric duct, responsible for the transport of semen later in life. This early stage of development is displayed in figure 3. During this early stage of development, testosterone secretion is not regulated by the LH and FSH, but by the peptide hormone chorionic gonadotropin (HCG) which is secreted by the placenta. HCG concentrations decline after the fourteenth week. LH and FSH can be detected from the tenth week of pregnancy onward. In some tissues testosterone is also converted into dihydrotestosterone (DHT) by the enzyme 5α-reductase. DHT induces the development of the prostate, the seminal vesicles and the bulbourethral glands (all partly responsible for the secretion of fluid that will form semen together with spermatozoa) during the tenth to twelfth week. DHT is also responsible for the formation of the male external genitalia: the penis and the scrotum, which are completely formed (but continue to grow) at the end of week fourteen. The testes start to descend into the scrotum in week 10 and between seven and nine months the testes are fully descended and fetal development of the male genital system is completed.

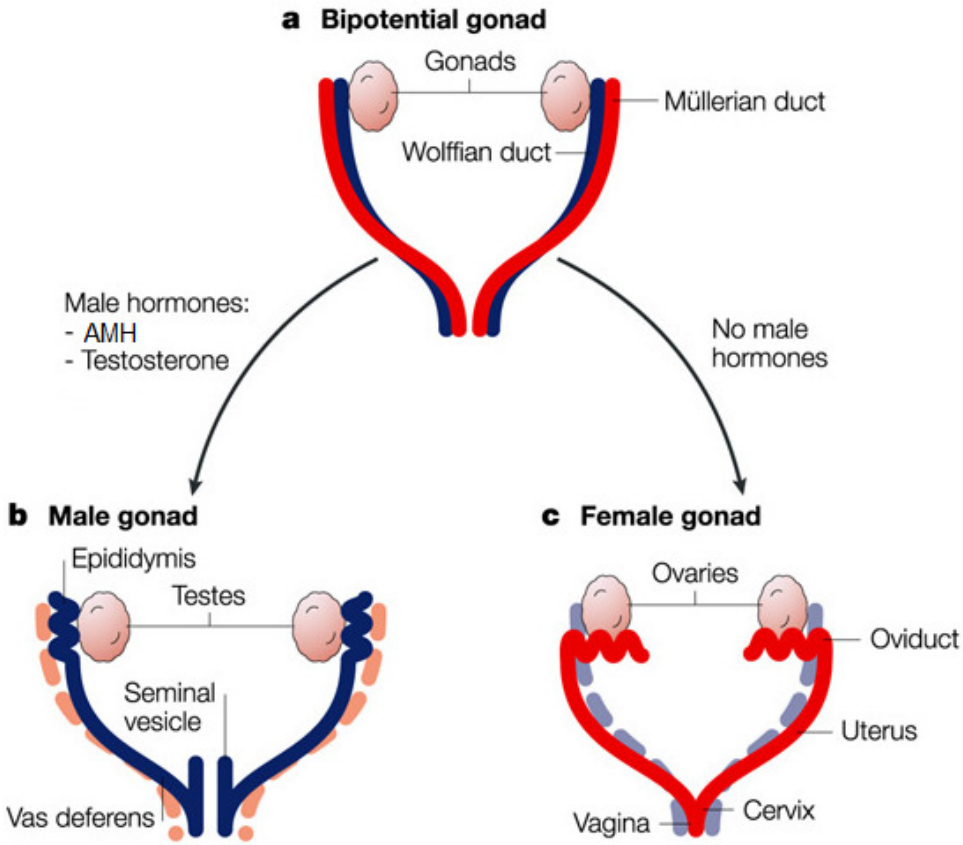

Fig. 3. Fetal development of the internal sex organs. (**a**) The indifferent gonads are accompanied by the mesonephric or Wolffian ducts and the paramesonephric or Müllerian ducts. At the beginning of the seventh week of fetal development, sexual differentiation occurs in either the male direction (**b**) due to the action of AMH and testosterone or in the female direction (**c**) due to the absence of these hormones. (Figure adapted from Kobayashi & Behringer (2003)).

Levels of testosterone vary along fetal development. Testosterone is produced in males from nine weeks onward. Testosterone serum levels are maximal from the fourteenth to sixteenth week of male fetal development, when levels are comparable to testosterone levels observed in adult males. Due to involution of the Leydig cells from 24 weeks onward, circulating testosterone levels decrease progressively to levels observed in female fetuses. At birth though, testosterone levels in male newborns are higher than in females. Estrogen levels slightly increase till the end of fetal life but are lower in male compared to female fetuses (Sajjad, 2010). At birth, testosterone, LH and FSH levels in the umbilical cord are low. This is due to the high levels of estrogens in the placenta, which suppress the hypothalamus and pituitary. After birth however, the estrogen levels drop (due to the removal of the placenta) and continue to be low till the start of puberty in boys. In the first two weeks of life an increase in LH and FSH occurs which is followed by increases in testosterone levels and in the number of Leydig and Sertoli cells in normal male infants. Testosterone, LH and FSH levels continue to increase until three months of age, after which levels drop till the six month, and stable low concentrations are

maintained until puberty. This postnatal period of enhanced hormone levels and proliferation of Leydig and Sertoli cells is called the 'minipuberty' of infancy. Its hypothesized function is the activation of the HPG-axis and "priming" of the reproductive system (Lewis and Lee, 2009). Because the number of Sertoli cells is a determinant of spermatogenic potential, the postnatal increase in Sertoli cells might be important for the spermatogenesis during adulthood. The accompanying increase in gonadotropin and testosterone secretion leads to the proliferation of germ cells. Additionally, an increase in the transformation of gonocytes (primitive reproductive cells) to spermatogonia is present which is thought to be driven by testosterone. Gonadotropin stimulation of the testis during this developmental window, might be essential for Sertoli cell function and spermatogenesis later in life (Quigley, 2002).

Puberty is the transitional period between childhood and adulthood during which the secondary sex characteristics develop, the growth spurt occurs and the body becomes capable of reproduction. The onset of puberty varies, but usually starts around the age of twelve in boys. Puberty onset is characterized in both girls and boys by an increase in amplitude of GnRH pulses after a relative quiescent period and consequently by an increase in LH and FSH pulses. As a result the Leydig cells increase their testosterone production and maturation of spermatogenesis occurs, resulting in testicular enlargement. High levels of testosterone during puberty are mainly responsible for the induction of secondary sex characteristics in boys including penile enlargement, the development of a low voice. Conversion of testosterone to DHT induces body hair. The growth spurt in males is largely dependent of estradiol (converted from testosterone) though estradiol levels are lower than in women. The increased production of sex steroids and its precursors during puberty is not only the result of gonadarche, the reactivation of the HPG-axis, but also of the adrenarche, the increase in adrenal androgen secretion. In boys puberty is usually completed by the age of sixteen to eighteen. During adulthood testosterone levels remain high and only slightly decrease as men become older. Plasma testosterone levels lay normally around 20 nmol/l in men during adult life, whereas estradiol levels should be lower than 0.2 nmol/l (Grumbach, 2002).

3.3. Sex Steroids in the Development of the Female Reproductive System

In genetic females (XX) the Y chromosome containing the SRY gene is lacking and the SRY peptide is therefore not produced in the sex cord cells of the indifferent gonad. As a consequence, these cells do not differentiate into Sertoli cells, and AMH and testosterone are not produced, leading to the initiation of female development. In females, the primitive sex cords degenerate and secondary cortical sex cords develop. The primordial germ cells are stimulated by cells of the secondary cortical sex cords to differentiate into oocytes and follicle cells of the ovary. Since the mesonepric ducts require high levels of testosterone for development, they

degenerate in females. The paramesonepric ducts however do not degenerate due to the absence of AMH and give rise to the oviducts, uturus and superior vagina in female fetuses as is shown in figure 3. This process takes places from the start of the third month and is completed by the end of the sixth month of fetal development. The formation of the ovaries is also completed by the end of month six. Low levels of estradiol produced by the granulosa cells of the fetal ovaries support follicular maturation but have only a minor role in other aspects of prenatal sexual differentiation, because maternal estrogens reach the fetuses of both sexes. The development of external genitalia in the female fetuses starts at the eleventh week of development due to the absence of androgens and the presence of estrogens within the maternal system. By the end of week 20, the female external genitalia, i.e., the clitoris, vestibule of the vagina and the labia minora and majora are present. It thus appears that fetal female sexual differentiation is rather independent of hormonal stimulation. Testosterone is also present in the female fetus but levels are substantially lower than in males (Larsen et al., 2001).

At birth, estradiol, testosterone, LH and FSH levels in the umbilical cord are low in females as well, due to the high levels of estrogens in the placenta. After birth, the testosterone and estradiol levels drop even more, just as in males, and testosterone levels continue to be low during the rest of life and are also lower than in males. Minipuberty in females is different than in males. During the first two weeks LH and FSH also increase in the female infant, with a higher increase in FSH and a lower increase in LH compared to male infants. An increase in estradiol levels occurs during the first 2-4 months of life as a result of the FSH induced rapid maturation of ovarian follicles during the same period. This postnatal increase in estrogen levels and in the maturation of ovarian follicles is thought to be essential for ovarian function later in life (Quigley, 2002). After minipuberty, levels of LH, FSH and estradiol remain low until puberty (Grumbach, 2002).

In girls, the mean age of puberty onset is ten years. Increased GnRH, LH and FSH pulsatility stimulates follicular development of the ovaries leading to increased levels of estrogen during puberty. Estrogen-dependent tissues then respond leading to many sex characteristics including breast growth, the growth spurt and increased fat composition. As estrogen levels gradually rise a point is reached when the feedback to the hypothalamus and pituitary becomes positive, allowing the mid cycle LH surge necessary for ovulation, the hallmark for female sexual maturity. Levels of androgens including testosterone also increase during puberty in girls causing for example growth of pubic hair. In girls puberty is completed by the age of fifteen to seventeen (Divall & Radovick, 2009). Until the menopause levels of estrogens and androgens decline only slightly with age. During adulthood plasma testosterone levels lay around 2 nmol/l. whereas estradiol levels vary during the menstrual cycle between values of 0.1 and 0.8 nmol/l. in women. However, the period of menopause is characterized by a

dramatic fall in estrogen levels due to decreased function of the ovaries. As a consequence of the decreased negative feedback in menopausal and postmenopausal women, LH and FSH levels are very high and the menstrual cycle does not occur anymore (Burger, 1996).

3.4. Sex Steroids and Differentiation of the Sexual Brain

Testosterone and estrogens are thus essential for development of the male and female reproductive system. Though estrogens does not appear to have an important contribution to sexual differentiation of the genitals during fetal development, they do have a major influence on particularly female pubertal development. The effects of testosterone and estrogens during prenatal and neonatal development are called organizational effects. These effects are usually permanent and occur during a specified sensitive period. The effects of sex steroids during puberty are called activational effects, because they "activate" the structures and circuits that were created during early development (Swaab, 2004). Since testosterone and estrogens have such a major influence on development of the sex organs, they are also likely to reach the brain and contribute to sexual development of the brain. Actually, possible structural differences in the brain resulting from the interaction among sex hormones and the developing brain cells, are hypothesized to be the basis of a wide variety of sex differences such as gender identity (acting and feeling like either a man or woman), sexual orientation (hetero-, homo-, or bisexuality) and related behavior. Disturbances between the interaction of sex steroids and the brain during pre- and neonatal development, the organizing period, are thought to permanently influence later behavior (Bao & Swaab, 2010).

3.4.1. Gender Identity & Transsexuality

Development of the brain however, takes place later in development than development of the sex organs. Sexual differentiation of the brain is thought to start during the second half of pregnancy and continues upon reaching adulthood. This indicates that both processes, i.e. sexual differentiation of the gonads and sexual differentiation of the brain may be influenced independently by sex steroids. Indeed, the occurrence of transsexuality is thought to demonstrate that these processes occur independently. Transsexuals are either people with male sexual organs and a male body who nevertheless experience their identity as females, or people with female sexual organs and a female body who experience their identity as males (Swaab, 2010). Transsexuality is the most extreme gender-identity disorder and postnatal social factors cannot be responsible for this disorder (Coolidge et al., 2002). The exact cause of transsexuality is still unclear but sex steroids during fetal development are thought to play an important role in the development of this disorder. This is supported by the increased prevalence of transsexuality among CAH girls. Moreover, children of epileptic women who took

phenobarbital or diphantoin during their pregnancy, chemicals which change the metabolism of sex steroids leading to decreased levels of testosterone, have an increased risk of transsexuality (Dessens et al., 1999). Sex steroid levels, particularly testosterone, thus seem to have an important organizational influence on gender identity. Distortion of these levels might lead to the development of transsexuality.

This assumption points to possible differences in the brain between men, women and transsexuals. These structural differences were hypothesized to be present in certain hypothalamic and limbic structures, since they are involved in sexual behavior in mammals. Indeed, differences have been found in the bed nucleus of the stria terminalis (BSTc) and in the interstitial nucleus of the anterior hypothalamus-3 (INAH-3), areas influenced by early testosterone exposure. In humans, both the BSTc and INAH-3 are twice as large in males compared to females (Kruijver et al, 2000; Garcia-Falgueras & Swaab, 2008). Moreover, the BSTc and INAH-3 in male-to-female (MtF) transsexuals have been found to have a similar volume as in women, whereas in female-to-male (FtM) transsexuals the BSTc and INAH3 have a male volume (Kruijver et al, 2000; Garcia-Falgueras & Swaab, 2008). Hence the above evidence regarding transsexuality demonstrates that sex steroids have important prenatal or organizational effects. In addition, this evidence thus provides additional support, next to disorders of sex steroids such as CAH and CAIS, that sex steroids, especially testosterone, are important in the sexual differentiation of the brain and (sexual) behavior.

3.4.2. Behavioral Sexual Differentiation

The evidence discussed so far is in line with traditional views of sexual differentiation and suggests that the male genitals and brain develop as a consequence of the presence of androgens, mainly testosterone, secreted by the male's testes, whereas the female genitals and brain develop mainly in the absence of these hormones. However, more recent evidence from animal studies questions the relative absence of estrogen action in the developing fetal brain (Bakker and Brock, 2010). Bakker et al. (2006) created a mouse model in which the a-fetoprotein (AFP) gene was knocked out. AFP is present in fetal blood and can bind estradiol. The female AFP-KO mice were clearly defeminized (lost the capacity to show female-typical sexual behaviors) since they did not show lordosis behavior, which is an important feature of female sexual behavior in mice. When an aromatase-blocker (prevents conversion of testosterone to estradiol) was given during fetal life to AFP-KO female mice, they did show normal sexual behavior. Bakker et al. (2006) demonstrated with this experiment that AFP prevents estradiol from entering the brain and prevents the induction of defeminization in (fetal) female mice. These effects can normally occur in the male brain in response estradiol which is locally produced in the brain from testosterone after it has been taken up from

circulation. Testosterone can enter the fetal brain, whereas estradiol cannot due to action of AFP. Hence this evidence indicates that estradiol has a prenatal defeminizing and to some extend masculinizing effect.

However, other experiments show that estradiol does also have feminizing effects. Aromatase knockout (ArKO) mice and female rats treated postnatally with tamoxifen, an estrogen receptor antagonist, show less lordosis behavior. But when these ArKO mice are treated with estrogen postnatally, they do show normal lordosis behavior (Bakker et al., 2002). These results indicate that estradiol is important for the differentiation of female sexual behavior as well, though only postnatally. Due to the absence of AFP and the estrogen production by the ovaries, substantial levels of estradiol can enter the brain after birth.

These results combined with evidence from previous experiments, indicate that for the differentiation of male sexual behavior high levels of testosterone and estradiol are required during the prenatal period followed by an increase in testosterone levels during minipuberty and puberty. For the normal differentiation of female sexual behavior on the other hand, estradiol and testosterone levels are expected to be low prenatally followed by an increase in estradiol levels during the postnatal period and puberty. This hypothesis of how estradiol can have both feminizing and defeminizing effects is displayed in figure 4.

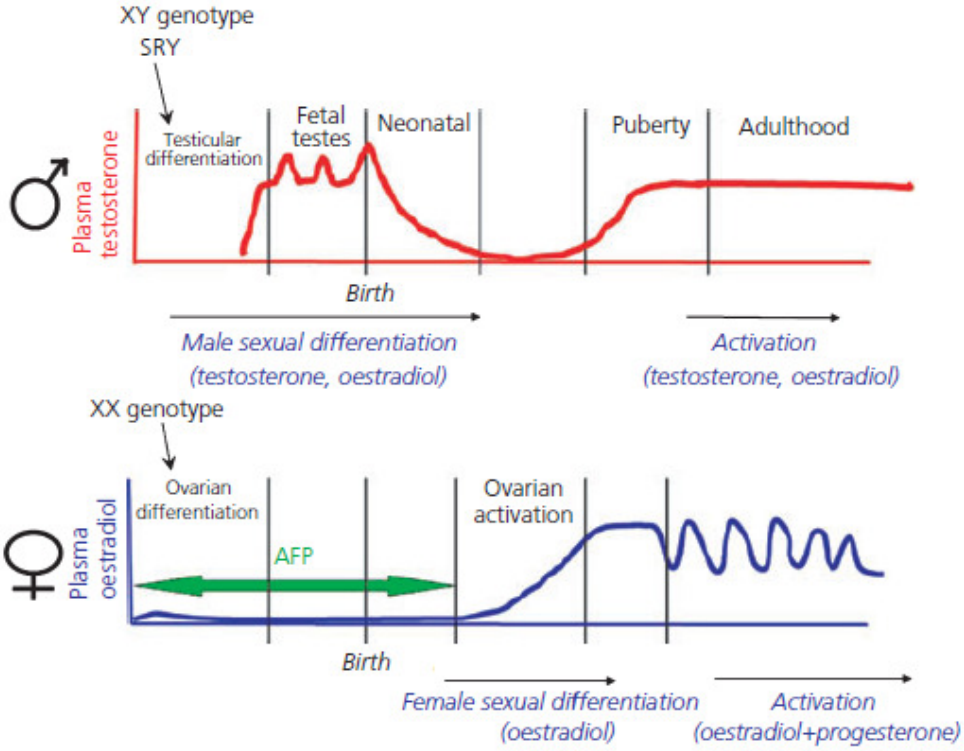

Figure 4. Hypothesis of the defeminizing and feminizing effects of estradiol during development. Male-typical sexual behavior is hypothesized to occur prenatally in genetic males under the influence of testosterone and estradiol, which is avoided in fetal genetic females by the neuroprotective actions of a-fetoprotein (AFP). Female typical sexual behavior probably occurs postnatally in genetic females under the influence of estradiol produced by the ovaries and the absence of AFP. (Figure adapted from Bakker & Brock (2010)).

Evidence regarding the role of estradiol in differentiation of human sexual behavior is unfortunately lacking, but similar mechanisms of sex steroid actions are expected to be present in humans. It is also not precisely known by which structures in the brain estradiol exerts its effects on behavioral sexual differentiation. However, the limbic system is thought to be important sexual behavior. In humans estrogen receptors have been found in the hippocampus and amygdala. Evidence from animal studies indicates that estrogen receptors are also present in several hypothalamic areas (Martin & Behbehani, 2006). Therefore estradiol might exert its effects on behavioral sexual differentiation via these structures in humans as well, although direct evidence for this assumption has not yet been found.

3.4.3. Sexual Orientation

Sex steroids are also implicated in the development of sexual orientation. Sexual orientation refers to which gender (male or female) a person is attracted. This can be to the opposite sex (heterosexual), to the same sex (homosexual) or to both sexes (bisexual). The apparent impossibility to change someone's sexual orientation (LeVay, 1991), excludes the option of societal or environmental causes and indicates biological mechanisms. Though family and twin studies have indicated that genes contribute 50% to the development of sexual orientation, several studies also point to a role of sex steroids. One such an indication is the large proportion of CAH girls who are homo- or bisexual, indicating a role for testosterone (Hines et al, 2004). Other evidence comes from children of women who took the medicine diethylstilbestrol (DES) during their pregnancy. DES can be subscribed to prevent miscarriage. It is an synthetic estradiol, not able to bind AFP and can thus reach the prenatal brain of the female fetus. It has become evident that DES use during pregnancy, increased the chance of giving birth to a girl which is homosexual or bisexual (Ehrhardt et al., 1985). This strongly suggests a role of estradiol in determining sexual orientation. The combination of both studies indicates that a normal male partner preference in females is induced due to the absence of estradiol and testosterone action in the female prenatal brain, whereas the female partner preference in males in induced due to the presence of estradiol action in the male prenatal brain. Abnormal estradiol and testosterone levels in either the male or female fetal brain may thus lead to homosexuality or bisexuality. The important role of estradiol and testosterone in sexual orientation or partner preference is also supported by evidence from animal studies. Male rats who received a aromatase blocker, showed a male instead of the normal female partner preference (Bakker et al., 2002).

Because sexual orientation is hypothesized to be the result of interactions between sex steroids and the brain, structural brain differences are also expected to be present between people who differ in sexual orientation. An example is the INAH-3, which was found to be twice as small in heterosexual women and homosexual men compared to heterosexual men (LeVay,

1991). The anterior commissure, which connects the amygdalae and hippocampi, and known to be larger in women than in (heterosexual) men, was found to be larger in homosexual men than in heterosexual men. No differences were found with respect to the BSTc (Allen & Gorski, 1992). Unfortunately, evidence of a direct influence of sex steroids on these structures is lacking. However, studies regarding sexual orientation indicate that sex steroids are likely to contribute significantly and that structural differences are present in the brain between people who differ in sexual orientation.

Ample evidence thus supports the importance of the testosterone and estrogens in the sexual differentiation of the genitals and the brain. Disorders leading to abnormal levels of sex steroids such as CAIS and CAH as well as the occurrence of transsexuality and differences in sexual orientation do not only provide insights into the organizing effects of sex steroids in sexual differentiation but also indicate that specific critical time windows are present during which certain sex steroid actions need to occur to induce either male or female features of sexual differentiation. Contradictive to former research, relative new evidence also postulates a major role of estradiol next to testosterone, in male prenatal brain development. However, much more research is necessary because many questions regarding the role of sex steroids in sexual differentiation, such as how sex steroids induce specific structural differences in the brain, remain to be answered.

4. Non-Reproductive Effects of Testosterone on the Brain

Next to the effects of testosterone on reproductive behavior and sexuality, testosterone has also other effects on the brain. These effects can be seen in a wide variety of behavioral and cognitive domains. Prominent differences have been described between men and women regarding aggression, risk-taking and visuospatial abilities. Testosterone influences these domains. The effects of testosterone on aggression, risk-taking and visuospatial abilities will be discussed in this chapter. Subsequently, these effects will be used to explain the differences between men and women in these domains.

4.1. Testosterone and Aggression

Aggression comprises all behaviors that are hostile, destructive, and/or violent. It generally has the potential to inflict injury or damage to the target person or object. Aggressive behavior can be divided in reactive aggression and proactive aggression. Reactive aggression is characterized by anger, loss of control, impulsiveness and increased responsivity to threat. Proactive aggression on the other hand, includes premeditated social violence, and thus planned behavior.

Proactive aggression is usually not associated with a response to threat or with frustration (Siever, 2008).

The link between testosterone and aggression has been studied for more than 35 years. Testosterone has been implicated in mainly reactive aggression but also in proactive aggression. Testosterone has been shown to increase various aspects of aggressive and dominant behavior in many reptilian and mammalian species (Archer, 2006). In addition, castration of both animals and humans results in reduced aggression (van Goozen et al., 1995). Research in the field of human social behavior indicates that high testosterone levels are associated with increased anger, aggression, interpersonal and aggressive dominance and criminal violence. The link between aggression and testosterone seems to be more evident in nonhuman animals, which might be explained by the well developed prefrontal cortex in humans, as is discussed later in this paragraph (Mazur & Booth, 1998). Only few studies in humans have found evidence that testosterone within the normal range directly causes aggression (Archer, 2006). It is therefore more accurate to say that testosterone has been associated with aggression. Evidence for the association of testosterone and human aggression comes from various studies including clinical observations, correlation studies, and various administration, behavioral and imaging studies.

Several lines of research address the assumed role of testosterone in aggression. People with the psychiatric condition borderline personality disorder, which includes reactive aggression, have been shown to have high levels of plasma testosterone compared to people without this disorder. In addition also in patients with antisocial personality disorder, characterized by proactive aggression, high levels of testosterone are found (Räsänen et al., 1999). Since aggressive behavior is related to criminal violence, studies regarding aggressive behavior in prisoners have also been conducted. These studies have found that men with higher testosterone levels committed more violent crimes (against persons) than inmates with low testosterone levels. In addition male inmates with higher testosterone levels also violated more rules in prison (Dabbs et al, 1995). Similar associations of testosterone and violent behavior have been found in female prisoners (Dabbs & Hargrove, 1997).

Hormonal administration studies have shed some light on the influences of testosterone on aggression as well. Evidence from several studies showed that when testosterone was administered and testosterone levels increased, increases in anger, aggression and hostility and a decrease in the recognition of threat could be observed (O'Connor et al., 2004; Pope et al., 2000; Su et al., 1993; van Honk & Schutter, 2007). However, it must be noted that except for van Honk & Schutter (2007), these studies administered testosterone doses that lead to supraphysiological plasma levels of testosterone, which makes the biological relevance of this data somewhat questionable.

Overall though, the present evidence suggests that higher testosterone levels are associated with various aspects of aggressive behavior during adult life. This indicates that testosterone exerts its influence of aggression via activational effects. To date there is little evidence of any prenatal or postnatal effects of testosterone on aggression (Archer, 2006). The exact mechanisms by which testosterone exerts its activational effects on aggression are however subject of discussion. Questions also remain with respect to the relatively weaker link between testosterone and aggression in humans. The relative weaker association between testosterone and aggression in humans might be (partly) explained by the well developed prefrontal cortex (PFC) in humans. The PFC might partly suppress direct hormonal effects on behavior (Curley & Keverne, 2005). One area of the PFC, the orbitofrontal cortex (OFC) is thought to be involved in self-regulation and impulse control. Increased activity of the OFC has been shown to induce low levels of (reactive) aggression, while lesions of this brain region lead to impulsive behavior and hyper-aggression (Blair, 2004). The OFC has direct connections with the amygdala, a limbic structure involved in the processing of biological relevant stimuli. Its main role is thought to promote vigilance and arousal (Davis & Whalen, 2001). High activity of the amygdala has been found in patients with borderline personality disorder (Herpertz et al., 2001). Activity of the amygdala also increases when anger-provoking stimuli are shown (Hermans et al., 2008). Taken together, these results indicate that high activity of the amygdala combined with low activity of the OFC might promote (reactive) aggression.

Testosterone might therefore act on the amygdala and OFC to influence aggressive behavior. Indeed, neuroimaging studies have demonstrated that testosterone administration enhances amygdala activity (Hermans et al., 2008). Moreover, a dense population of neurons containing nuclear androgen receptors was found to be present in the amygdala. These neurons were activated by testosterone (Simerly et al., 1990). Furthermore, androgen receptors are present in the OFC as well (Finley & Kritzer, 1999), and testosterone levels have been found to correlate negatively with activity of the OFC in both men and women (Mehta & Beer, 2010). At last, a fMRI study showed that administration of testosterone decreased the functional coupling between the amygdala and OFC (van Wingen et al., 2009). Taken together, these results indicate that testosterone may reduce the regulatory control of the OFC over the amygdala, resulting in high activity of the amygdala and low activity of the OFC. High levels of testosterone are therefore likely to promote aggression. However, one should keep in mind that testosterone is not thought to be the only substance involved in aggression. Aggression modulating roles have also be proposed for serotonin and cortisol (van Honk et al., 2010).

In addition, recent evidence also suggests a possible role for estrogens, including estradiol in aggression. Some clinical trials involving estrogen treatment have provided insights in the modulating properties of estrogen on aggression in humans. Two studies demonstrated

that postmenopausal women receiving hormone replacement therapy (HRT) scored lower on hostility scales (a component of aggression), than women who did not use HRT (Steffen et al., 1999; Olson et al., 2004). Some elderly men and women diagnosed with dementia exhibit physical violence and verbal aggression. This behavior was found to correlate negatively with peripheral estradiol and positively with peripheral testosterone (Orengo et al., 2002). Moreover administration of estrogen to these patients resulted in a decrease of aggressive behavior (Kyomen et al., 1999). These studies indicate that estrogens might reduce aggressive behavior. Unfortunately, results from animal research are conflicting, with estrogen increasing aggression in some species, while decreasing aggression in other species (Trainor et al., 2006). Nevertheless, the evidence from human studies indicates that estrogen inhibit aggression. Estrogen receptors have been found on the OFC and amygdala (Martin & Behbehani, 2006), thus estradiol might exerts its effects on aggression via these structures as well.

Overall, the evidence indicates an aggression inducing effect of testosterone and an aggression inhibiting effect of estrogens in humans. Together the aggression-modulating effects of testosterone and estrogens might help to explain the differences in aggression between men and women. Men are known to show more aggressive behavior and commit more aggression-related crimes than women. This might be explained by the fact that men have ten times higher levels of testosterone than women. Testosterone is hypothesized to reduce the functional coupling between the OFC and amygdala, which might lead to reduced control over aggression. Therefore men are thought to show more aggressive behavior. Women on the other hand, have higher levels of estrogens, including estradiol which appears to inhibit aggressive behavior. The lower levels of testosterone and higher levels of estrogens in women, might explain why women show less aggressive behavior than men. The evidence thus clarifies the overall lower levels of aggression in women compared to men. However, the differences in testosterone and estrogen levels and action does not explain why men show relative more physical aggression whereas women show more indirect and verbal aggression. Perhaps this might be explained by other substances in the brain such as cortisol and serotonin or genetics. Nevertheless, much more research to aggression, including the role of the sex steroids, is necessary to understand the exact biological mechanisms of aggression.

4.2. Testosterone in Risk-Taking and Trust

Testosterone has also been related to risk-taking, a domain influence by reward sensitivity and fear. When a person's sensitivity for reward is high or fear is low, this may lead to increased risk-taking and disadvantageous decision making (van Honk et al., 2004). A clear difference between men and women is present with respect to risk-taking, with men, especially young men, showing

increased risk taking compared to women in for example, conflicts, outdoor activities and car driving (Flisher et al., 1996).

An experimental paradigm which assesses the balance between the sensitivity of punishment and reward is the Iowa gambling task. When reward sensitivity is high, disadvantageous decision making on the Iowa gambling task is observed. Van Honk et al. (2004) demonstrated that testosterone affects reward sensitivity by showing that testosterone administration in healthy young women induced an increase in reward sensitivity as reflected by disadvantageous decision making on the Iowa gambling task. Moreover, Stanton et al. (2010), recently showed that also endogenous testosterone was associated with risk-taking on the Iowa gambling task. Both men and women with high levels of testosterone made riskier choices compared to their low-testosterone counterparts of the same sex. Higher levels of testosterone are thus associated with greater risk-taking. In addition, testosterone administration also reduced the fear-potentiated startle in humans (Hermans et al., 2006). These observations are consistent with animal research showing an increase in reward sensitivity and reductions of social punishment and fear after testosterone treatment (Boissy & Bouissou, 1994).

With regard to financial risk taking, important associations with testosterone have been found as well. Coates and Herbert (2008) conducted a real-life experiment with traders on the stock-market during which testosterone levels were measured. They found that high levels of testosterone during the morning were associated with a higher profit that day, as a consequence of increased risk taking, compared to low morning levels of testosterone. Besides this activational effect, prenatal testosterone has also been found to contribute to (financial) risk-taking. A measure of prenatal androgen exposure is the digit ratio, with smaller ratios reflecting increased androgen exposure (Manning et al., 1998). Coates et al. (2009) demonstrated that the digit ratio predicted both the profit and loss of trader over a 20-month period as well as the number of years they had survived in the business. Lower digit ratios, reflecting increased prenatal testosterone exposure, were associated with increased risk-taking and higher profits. Hence, it appears that testosterone has both an organizational as well as an activational effect on risk-taking.

Via restraining interpersonal trust, testosterone might ensure social scrutiny for these economic concerns. Indeed testosterone administration has been shown to decrease interpersonal trust in humans, specifically in those who are highly trusting (Bos et al., 2010). This decrease in trust is therefore adaptive and might decrease trust to a more advantageous level for rational decision making which is necessary for successful trading on the stock market. At present, there is no evidence of a direct effect of estradiol on trust and risk taking in (healthy) humans (Zethraeus et al., 2009). Little knowledge is present to date about the neurobiological mechanisms whereby testosterone acts on interpersonal trust and risk-taking. Similar

mechanisms to aggression though, are thought to be involved. With regard to risk-taking and trust, testosterone is thought to decrease the control of the OFC over the amygdala as well, because administration of testosterone decreased the connectivity between the OFC and amygdala in response to threat (van Wingen et al., 2009). This shift toward the evolutionary older brain regions might put the brain in a defensive or vigilant mode, whereby interpersonal trust is down-regulated and risk-taking is increased. This vigilant state might be necessary for success in competition, including success in competition of traders on the stock market. However it might be necessary to reevaluate the exact relationship between testosterone and risk-taking in humans because success on the stock market cannot be established by unlimited risk-taking since it requires a balance between financial threat and reward (Bos et al., 2010).

Taking together the evidence indicates that high levels of testosterone increase risk-taking and reduce interpersonal trust in humans. The higher levels of testosterone in men, might provide an explanation for the increased risk-taking in (young) men compared to women. However, the exact relation between testosterone, risk-taking and trust remains to be defined.

4.3. Testosterone and Visuospatial Abilities

Another prominent difference between men and women which has been linked to testosterone function is visuospatial performance. Compelling evidence from many studies demonstrates that men outperform women on visuospatial tasks (Thilers et al., 2006). Sex differences regarding visuospatial abilities have been found on cognitive tasks involving spatial processing such as mental rotation, spatial navigation and spatial memory and sensorimotor tasks such as targeting (Aleman et al., 2004; Hines et al., 2003). One of the most used tasks to assess visuospatial abilities is the Vandenberg and Kuse mental rotation test (MRT) (Vandenberg & Kuse, 1978). For mental rotation the active manipulation of objects in mind is required which is based on visuo-spatial memory functions.

Though contradicting results have been found as well, evidence from many studies demonstrates an association between testosterone and performance on visuospatial tasks. As men get older both testosterone levels and cognitive performance, including mental rotation, decline (Janowsky, 2006). This observation indicates a possible relation between testosterone and visuospatial performance. Indeed, linear relationships between testosterone and visuospatial performance have been found in older men, who performed better when testosterone levels were higher (Thilers et al., 2006). Further support comes from testosterone and anti-testosterone supplementation studies in older men and male-to-female transsexuals, respectively. These studies demonstrated that increasing the low levels of circulating testosterone in older men improved their performance on spatial tasks (e.g. Cherrier et al., 2001), whereas anti-androgen treatment in male-to-female transsexuals resulted in a significant

decrease in performance (Slabbekoorn et al., 1999). Moreover, a single testosterone administration in women improved performance on the MRT as well (Aleman et al., 2004) and an increase in performance on the MRT was also found in female-to-male transsexuals receiving androgens (Slabbekoorn et al., 1999). However, in some studies with younger men the relation between endogenous testosterone and visuospatial abilities was absent or even negative (e.g. Martin et al., 2008). This might be explained though by the presence of a curvilinear relationship, with very high testosterone levels leading to stagnation of performance or a lower performance (Muller et al., 2005). The lower visuospatial performance, as a result of the low testosterone levels in women and older men, might however be open for improvement by increasing the testosterone levels.

The discussed evidence thus provides evidence for an important activational role of testosterone in visuospatial abilities. However, some other studies indicate possible prenatal effects of testosterone on visuospatial performance, suggesting a possible organizational role of testosterone as well. With regard to this prenatal influence of testosterone though, the picture is less clear. Hines et al. (2003) found no advantage on mental rotation in CAH girls, but did find a targeting advantage. Another study did however report a significant association between the digit ratio and mental rotation, with lower ratios (which reflect higher androgen exposure) resulting in better performance on mental rotation in men (Manning & Taylor, 2001). Similar associations between testosterone and visuospatial performance were found by some other studies (e.g. Falter et al., 2006). Although more research is necessary, the current evidence seems to indicate both an organizational and an activational role of testosterone in the development of visuospatial abilities.

A brain structure found to be important in both humans and animals in visuospatial tasks, specifically spatial navigation and memory, is the hippocampus (Geinisman et al., 1995). Patients with lesions of the hippocampus have been shown to suffer from deficits in these domains. Androgen receptors and androgen receptor mRNA are highly expressed in this brain area (Beyenburg et al., 2000). Testosterone might therefore act on the androgen receptors in the hippocampus to affect spatial abilities. In addition, several cortical areas, including the parietal cortex, have also been implicated in visuospatial abilities as determined by fMRI research (Kucian et al., 2007). More research is necessary to define the exact influences of testosterone on the hippocampus and cerebral cortex and the consequential effects on visuospatial abilities.

In addition, some research also indicates a possible influence of estradiol on the performance of the mental rotation task and thus visuospatial abilities. Hausmann et al. (2000) showed that women at the low estrogen phase outperformed women at the high estrogen phase in the menstrual cycle. Moreover estradiol levels were found to correlate positively with the reaction time on the mental rotation task, suggestive that estradiol might decrease the

performance of the mental rotation task by influencing the perception of the rotation process (Kozaki & Yasukouchi, 2008). Since estrogen receptors have been found in the hippocampus and the frontal cortex, estradiol might exert its influences on visuospatial abilities via these structures as well (Martin & Behbehani, 2006).

To summarize, most evidence suggests that testosterone enhances visuospatial performance. In addition, there are also a few indications that estradiol might reduce visuospatial performance. Taken together, this evidence might provide an explanation for the enhanced visuospatial abilities of men compared to women. The higher levels of testosterone in men likely increase the performance on visuospatial tasks, whereas in women the higher estradiol levels might reduce visuospatial abilities. However, more research is necessary to provide insights into the mechanisms whereby testosterone and also estradiol exert their influence on visuospatial abilities.

Ample evidence thus supports the importance of testosterone in non-reproductive behaviors and cognitive functions such as aggression, risk-taking and visuospatial abilities. Evidence from correlation, administration and clinical studies has provided insights into the effects of testosterone on these functions and in the related differences between men and women. However, the exact mechanisms by which testosterone exerts its effects on aggression, risk-taking and visuospatial performance are only partly determined. Moreover, testosterone is likely to be not the only factor involved and higher levels of testosterone do not necessarily have to account for all the differences between men and women. Future research should address the exact neurobiological mechanisms and interactions by which testosterone exerts its effects on the described domains.

5. Non-Reproductive Effects of Estrogens on the Brain

Just as testosterone, estrogens have also non-reproductive effects on brain function. This chapter will focus on the effects of estrogens on memory. Prominent differences are present between men and women regarding verbal episodic memory and estrogens are thought to play an important role in it. Most of the described research focuses on the effects of estradiol.

5.1. Estrogens in Verbal Episodic Memory

The first indications for the effects of estrogens, in particular estradiol on memory were provided by observations of decreases in memory function in women after the menopause, when estradiol levels were reduced. Several studies reported correlations between estradiol levels and memory function, with lower estradiol levels predicting lower memory function (e.g. Wolf & Kirschbaum, 2002). Estradiol has been specifically implicated in verbal episodic memory.

Episodic memory is defined as the memory of autobiographical events, that is times, places and other contextual knowledge that can be explicitly stated. The part of episodic memory which involves information that can be verbally encoded and retrieved is called verbal memory. Paradigms to assess verbal episodic memory include paired associate learning and word-list learning tasks (Tulving, 1984). Wolf and Kirschbaum (2002) measured endogenous sex steroid levels and verbal memory (as assessed by paired associated learning) in elderly women and found a positive correlation between estradiol and verbal memory. Other studies found that high estradiol levels were associated with better delayed verbal memory and retrieval efficiency (Drake et al., 2000). Hogervorst and colleagues (2004) reported a positive correlation between plasma estradiol levels and verbal list recall in elderly women. Associations between estradiol and verbal memory were also found over the different stages of the menstrual cycle in younger women. Verbal memory was found to be better during the late follicular phase when estradiol levels are higher compared to the late luteal phase (Protopopescu et al., 2008).

Research regarding the potential benefit of estrogen treatment on episodic verbal memory function however has provided inconsistent results. Clinical results come from research to the effects of HRT on memory function in postmenopausal women and to the effects of estrogen treatment on episodic memory function in Alzheimer's disease. Some epidemiological studies in postmenopausal women have found that women on HRT perform better on several cognitive domains (including memory) than postmenopausal women without HRT (Hogervorst et al., 2000). Various placebo controlled studies testing the effects of estrogen treatment on verbal memory reported effects (e.g. Wolf et al., 1999), whereas others found no improvement. Results from a beneficial study by Yaffe et al. (2000) indicated that higher levels of free estradiol were associated with a lower risk of cognitive decline, including episodic verbal memory impairment. In addition, HRT appears to decrease the risk for dementia in postmenopausal women, thought results are inconsistent (Hogervorst et al., 2000). An explanation for the inconsistent results might be that HRT differs between studies. Some types of HRT do not contain estradiol, but synthetic estrogens instead, which might not be able to affect memory (Brinton et al., 1997). In line with this assumption are the results of a clinical trial in which midlife women with surgically induced menopause were included. This study reported a significant increase in verbal memory as a consequence of treatment with intramuscular estradiol (Maki et al., 2007). Since verbal episodic memory is affected in Alzheimer's disease, studies have also assessed the effects of estrogen treatment in these patients. Results of these studies are comparable with the effects of HRT in (post)menopausal women. Some studies report increases in episodic verbal memory performance or a protective effect of estrogen treatment on the risk of developing Alzheimer's disease later in life, whereas others did not report such effects (for a review, see Henderson, 2009).

The hippocampus and surrounding medial temporal lobe have been shown to be essential for episodic memory encoding. The first indications came from the observation of severely impaired long-term episodic memory after bilateral surgical removal of the hippocampus and surrounding areas, while other forms of memory remained relatively intact (Corking, 1984). Alzheimer's disease is characterized by atrophy which affects the hippocampus as well as cerebral regions. Atrophy of the hippocampus, most severely the CA1 region, and the surrounding medial temporal cortex plus prefrontal regions is thought to induce deficits in episodic memory in these patients. Estradiol likely exerts its influence on episodic memory via the CA1 region of the hippocampus. Estrogen receptors are profoundly present in this region and animal research has demonstrated that estradiol (together with progesterone) increases the numbers of dendritic spines on pyramidal neurons, leading to the formation of excitatory synapses (Woolley & McEwen, 1993). In addition, estradiol promotes survival of new neurons and enhances longterm potentiation in the hippocampus, a process thought to be necessary for the encoding of episodic memories (Foy et al., 2000).

Imaging studies can be used to investigate the possible beneficial effects of estrogens on hippocampal and cerebral activity. Two position emission tomography (PET) studies showed that estrogen therapy increased the activity of the hippocampus and regions of the prefrontal cortex during the retrieval stage which led to improvement of episodic memory (Resnick et al., 1998; Maki & Resnick, 2002). A fMRI study demonstrated that estrogen increased the activity of the right hippocampus during verbal retrieval (Gleason et al., 2006). The difference of the results between the PET and fMRI study might be explained by the lower sensitivity of fMRI versus PET. PET studies during resting state in Alzheimer's disease provide additional information. One study showed that women receiving estrogen therapy showed increases in metabolism in the lateral temporal regions, whereas in women without estrogen therapy no changes were observed (Rasgon et al., 2001). Another PET study showed that women who did not receive estrogen therapy had increased activity of the frontal and temporal regions compared to women with Alzheimer's diseases, whereas women with estrogen therapy showed the highest activity of the three groups in frontal and temporal regions (Eberling et al., 2000). In a study by Eberling et al. (2004) it was shown that estrogen users had increased metabolism in the frontal and temporal compared to nonusers. Together these PET studies show that estrogen might help to preserve brain function in the areas important for verbal episodic memory, which are affected by Alzheimer's disease.

Various studies have also assessed a possible influence of testosterone on verbal memory in men and women. Diverse outcomes have been reported, with some studies reporting positive and others finding negative associations. Absence of a link between testosterone and verbal episodic memory has also frequently been reported (for a review see Nelson et al., 2008).

The relation between testosterone and verbal episodic memory therefore remains inconclusive. Overall, whit respect to estrogens, the results indicate that estrogens, specifically estradiol, have a positive activational effect on verbal episodic memory, and most likely act via the hippocampus as well as frontal and temporal regions.

With respect to verbal episodic memory differences are present between men and women, with women having a better verbal episodic memory. The higher estrogen levels in women might partly explain why women have a better episodic verbal memory than men. In addition, the hippocampus is larger and contains more estrogen receptors in females which might lead to an increased sensitivity for estrogens in females compared to males (Mitterling et al., 2010). Combined, these results provide an explanation for the better verbal episodic memory in women. Moreover, most studies also show that women have an increased risk of Alzheimer's disease, in which verbal episodic memory is severely affected (Letenneur et al., 2000). Since estrogen levels are relatively high and the sensitivity for estrogens appears to be larger in women, women might be more dependent on the estrogens for episodic verbal memory function. After menopause, the large decrease in estrogen levels might lead to a large reduction in the number of excitatory synapses, a reduction in the number of neurons and a reduction of longterm potentiation in the hippocampus. This may eventually result in memory loss and in addition, it might make women more prone to Alzheimer's disease then men, in which sex steroid levels decrease gradually. However, it must be noted that this hypothesis is highly speculative. In addition, the assumptions made regarding estrogen and verbal episodic memory, have to interpreted with caution because most results come from studies which have been performed in postmenopausal women while evidence in younger women and men is lacking.

To summarize, estrogens, specifically estradiol, have been shown to have an important modifying function in verbal episodic memory. Evidence from various studies has provided insights into the mechanisms by which estrogens affect this function of the brain and into the related differences between men and women. However, many of the exact mechanisms by which estrogens exert their effects remain to be determined. Future research should focus on the exact neurobiological mechanisms by which estrogens influence verbal episodic memory. In addition, a distinction should be made between endogenous estrogens and synthetic estrogens, since they appear to have different effects.

6. General Discussion

The literature reviewed in this thesis shows that estrogens and testosterone have important influences on human sexual differentiation. Not only are these sex steroids essential for the sexual differentiation of the reproductive system, they also have important effects on the sexual differentiation of the brain. The influences of estrogens and testosterone on sexual brain differentiation are reflected by differences between men and women in several neurobiological, behavioral and cognitive domains. Organizational effects of testosterone and estrogens, taking place during the prenatal and neonatal period are thought to result in permanent structural brain changes and are likely to be essential for some core aspects of male and female differentiation, i.e. gender identity, sexual orientation and sexual behavior. Distortion of the interaction between sex steroids and the brain during critical time periods in early life is considered to permanently affect later behavior. Evidence for these assumptions is provided by animal studies and by disorders of sex steroids such as CAH and CAIS, which lead to aberrant (later) behavior. In addition, transsexualitity is also thought to result from distortion of sex steroid action during the prenatal period. However, many questions remain regarding the exact mechanisms by which testosterone and estrogens exert their effects on the brain.

Several sex differences in behavior and cognition have specifically been attributed to the higher testosterone levels in men compared to women. In this thesis the influence of testosterone on aggression, risk-taking and visuospatial abilities was discussed. Levels of aggression and risk-taking are higher in men and visuospatial performance is higher in men compared to women as well. Testosterone has been repeatedly demonstrated to influence these domains, mainly via activational effects. Risk-taking and aggression are thought to be mediated via the influence of testosterone on the OFC and amygdala, whereas visuospatial abilities are considered to be affected by testosterone action on the hippocampus and frontal cortex. Though higher levels of testosterone in men likely explain the sex differences in these functions, the exact mechanisms by which testosterone influences these functions are not completely clear. Furthermore, sex steroid levels in blood do not necessarily reflect the levels of sex steroids in the brain. The enzyme aromatase is present in the brain and might locally convert testosterone to estradiol. It could thus be the case that some of the effects assumed to be mediated by testosterone are in fact mediated by the action of estradiol (Bakker & Brock, 2010). Further research is necessary to provide more clarity about the exact mechanisms of testosterone action in the brain.

Estrogens have been implicated in several sex differences in cognition and brain function as well. The majority of studies have focused on the activational effects of estradiol, which is thought to be the most important estrogen. In this thesis the effects of estrogens on verbal episodic memory were discussed. Higher estrogen levels in women compared to men are

assumed to contribute significantly to sex differences in verbal episodic memory, with women outperforming men. The higher levels of estrogens in women are hypothesized to account for the improved performance in women by acting on the hippocampus. The use of different types of estrogens, i.e., synthetic estrogens and (endogenous) estradiol, in studies might possibly provide an explanation for the inconsistent results. Future research is necessary to determine the exact neurobiological mechanisms of estrogen action and should distinguish between the different types of estrogens.

Research to the influences on sex steroids on the brain in humans is however limited. Evidence in humans mainly comes from clinical observations (i.e., "experiments of nature") and administration studies. In these studies the brain is exposed to a combination of both androgens and estrogens, which makes it difficult to determine the exact individual contribution of a particular sex steroid. In addition, sex steroids can also be locally converted in the brain into other sex steroids, which makes the situation even more complex (Bakker et al., 2006). Furthermore, testosterone and estrogens are not the only factors involved. Various neurotransmitters and genetic influences, which have not been discussed in this thesis, are likely to affect the described functions as well (Arnold et al., 2004). For example, serotonin and cortisol have been implicated in aggressive behavior, next to testosterone (Van Honk et al., 2010). Similarly, additional factors are thought to be involved in the other described brain functions (Arnold et al., 2004).

Research to the influences of sex steroids on the human brain, will also in the future be largely restricted to "experiments of nature" and administration studies because of ethical reasons. Animal experiments might provide additional insights, yet it is difficult to determine to which extend these findings can be applied to humans, especially with regard to advanced cognitive functions. However, a possible option for future research is to determine the effects of sex steroids on the brain in patients who receive hormonal treatment. One patient group in which the effects of sex steroids can be investigated are women with polycystic ovary syndrome (PCOS). PCOS is a prevalent endocrine disorder associated with chronic hypersecretion of androgens (Legro, 2003). Symptoms include polycystic ovaries, oligomenorrhea and acne. Women with PCOS show a decreased performance on certain female favoring tasks, such as verbal episodic memory and an increased performance on certain male favoring tasks, including block tapping, compared to healthy female controls (Schattmann & Sherwin, 2007). These differences are thought to result from the increased testosterone levels. Women with PCOS commonly undergo anti-androgenic treatment. To learn more about the (absence of the) influence of androgens on cognition and to validate the previous hypothesis, the effects of anti-androgenic treatment on behavior and cognition in women with PCOS can be determined.

Further insights might be provided by investigating the effects of sex steroids in transsexuals undergoing cross-sex steroid treatment (Sommer et al., 2008). These transsexuals will undergo a blockade of their endogenous gonadal sex hormone production and will receive either estrogen treatment (male-to-female transsexuals) or androgen treatment plus an aromatase inhibitor (female-to-male transsexuals). Subsequently the individual activational effects of androgens and estrogens on cognition and brain activity can be determined.

The exact effects of the above described hormone treatment in PCOS and transsexuals can be investigated by administering tasks which assess behavior and cognition before and after treatment. At the same time the effects of sex steroids on brain activity can be determined when participants perform the behavioral and cognitive tasks while laying in the MRI-scanner. To assess the influences of sex steroids on aggression and risk-taking, aggression questionnaires, and the Iowa gambling task can be administered. To assess visuospatial performance and verbal episodic memory, the mental rotation task and paired associate learning or word-list learning tasks respectively, can be used. Based on the findings described in this thesis, testosterone administration in transsexuals (in combination with a blockade of estrogen) is expected to induce increases in aggression, risk-taking and visuospatial performance, whereas anti-androgenic treatment in women with PCOS will lead to the opposite effects. Estrogen treatment in male-to-female transsexuals (in combination with a blockade of androgens), is hypothesized to result in better verbal episodic memory. The fMRI results might in addition provide more clarity on the brain areas involved.

The above described studies will likely add to the current knowledge of the influences of sex steroids on the brain. However, many of the exact neurobiological mechanisms by which sex steroids influence brain functions, including interactions with other chemicals in the brain, still need to be determined.

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