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CHAPTER 4

Sexual differentiation of the human brain in relation to gender identity and sexual orientation

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Abstract: It is believed that during the intrauterine period the fetal brain develops in the male direction through a direct action of testosterone on the developing nerve cells, or in the female direction through the absence of this hormone surge. According to this concept, our gender identity (the conviction of belonging to the male or female gender) and sexual orientation should be programmed into our brain structures when we are still in the womb. However, since sexual differentiation of the genitals takes place in the first two months of pregnancy and sexual differentiation of the brain starts in the second half of pregnancy, these two processes can be influenced independently, which may result in transsexuality. This also means that in the event of ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain.

There is no proof that social environment after birth has an effect on gender identity or sexual orientation. Data on genetic and hormone independent influence on gender identity are presently divergent and do not provide convincing information about the underlying etiology. To what extent fetal programming may determine sexual orientation is also a matter of discussion. A number of studies show patterns of sex atypical cerebral dimorphism in homosexual subjects. Although the crucial question, namely how such complex functions as sexual orientation and identity are processed in the brain remains unanswered, emerging data point at a key role of specific neuronal circuits involving the hypothalamus.

Keywords: Gender identity; Homosexuality; Human brain; Sexual orientation; Sexual differentiation; Transsexuality

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General concepts

Gender identity and sexual orientation represent two fundamental functions in human neurobiology. These functions have hitherto mainly been discussed in relation to the specific signs of sexual dimorphism in the brain and the potential mechanisms thereof. By mapping differences between men and women in cerebral anatomy, function, and neurochemistry, neuroscientists are trying to identify sex typical and sex atypical actors in transsexual and homosexual individuals. This has been done in postmortem analyses of the brain, and investigations of neuronal anatomy, connectivity, and function by means of positron emission tomography (PET) and magnetic resonance imaging (MRI). The extracted networks are then mapped onto those known to be related to sexual behavior in animals to formulate biological underpinnings of homo- and transsexuality in humans. This widely used approach has several difficulties with this approach: (1) gender identity cannot be investigated in animals; (2) sexual behavior in animals is reflex-like and cannot simply be translated to sexual orientation and attraction in humans; (3) reliable sex differences in the human brain require investigations of large populations and have only recently been demonstrated reliably; (4) the majority of studies on sex differences do not account for sexual orientation of the investigated participants; (5) studies of homo- and transsexual persons are very limited, and only few comparisons have hitherto been presented between homo- and transsexual subjects.

An alternative and parallel approach is pinpointing the specific neuronal networks related to gender identity and sexual orientation, analyzing the factors programming these networks and possible differences between control, homo-, and transsexual subjects. Emerging fMRI and PET studies suggest that sexual arousal is mediated by specific core neuronal networks, which may be also involved in sexual orientation.

Sexual organization and activation of the human brain

The process of sexual differentiation of the brain brings about permanent changes in brain structures and functions via interactions of the developing neurons with the environment, understood in its widest sense. The environment of a developing neuron is formed by the surrounding nerve cells and the child's circulating hormones, as well as the hormones, nutrients, medication, and other chemical substances from the mother and the environment that enter the fetal circulation via the placenta. Along with the genetic code, all these factors may have a lasting effect on the sexual differentiation of the brain.

The testicles and ovaries develop in the sixth week of pregnancy. This occurs under the influence of a cascade of genes, starting with the sex-determining gene on the Y chromosome (*SRY*). The production of testosterone by a boy's testes is necessary for sexual differentiation of the sexual organs between weeks 6 and 12 of pregnancy. The peripheral conversion of testosterone into dihydrotestosterone is essential for the formation of a boy's penis, prostate, and scrotum. Instead, the development of the female sexual organs in the womb is based primarily on the absence of androgens (Swaab et al., 2003).

Once the differentiation of the sexual organs into male or female is settled, the next thing that is differentiated is the brain, under the influence, mainly, of sex hormones on the developing brain cells. The changes (permanent) brought about in this stage have organizing effects; later, during puberty, the brain circuits that developed in the womb are activated by sex hormones. This paradigm of sexual differentiation of the brain was coined by Phoenix et al. (1959) and has dominated the view on cerebral sex dimorphism during the last decades.

The fetal brain is protected against the effect of circulating estrogens from the mother by the protein α-fetoprotein, which is produced by the fetus

and binds strongly to estrogens but not to testosterone (Bakker et al., 2006, 2008). However, not only estrogens reach the brain via circulation, but the brain itself is capable of producing estrogens. In human beings testosterone may thus not only have a direct effect on a masculine brain, but, once converted into estrogens by aromatase, may also act on developing neurons. In addition, there are sex differences in brain steroid receptor distribution not only in adulthood (Ishunina and Swaab, 2008; Kruijver and Swaab, 2002; Kruijver et al., 2001; Swaab et al., 2001) but also during development (Chung, 2003), which may be genetically determined. In addition, in rat hormone receptor genes a sex difference in methylation pattern occurs during development (Schwarz et al., 2010). In rats, the formation of estradiol in the brain by aromatization of circulating testosterone is the most important mechanism for virilization of the brain (Gorski, 1984), but, as seen below, it does not determine human gender identity or sexual orientation.

There may also be direct genetic effects that affect the sexual differentiation of the brain without involving the sex hormone receptors.

Sex hormones and human brain development

During fetal development, the brain is influenced by sex hormones such as testosterone, estrogens, and progesterone (Swaab, 2004). From the earliest stages of fetal brain development, many neurons throughout the entire nervous system already have receptors for these hormones (Chung, 2003). The early development of boys shows two periods during which testosterone levels are known to be high. The first surge occurs during mid-pregnancy: testosterone levels peak in the fetal serum between weeks 12 and 18 of pregnancy (Finegan et al., 1989) and in weeks 34–41 of pregnancy the testosterone levels of boys are ten times higher than those of girls (De Zegher et al., 1992; Van de Beek et al., 2009). The second surge takes place in the first three months after birth. At the end of pregnancy, when the α-fetoprotein level declines, the fetus is more exposed to estrogens from the placenta, this exposure inhibiting the hypothalamus–hypophyseal–gonadal axis of the developing child. Loss of this inhibition once the child is born causes a peak in testosterone in boys and a peak in estrogens in girls (Quigley, 2002). The testosterone level in boys at this time is as high as it will be in adulthood, although a large part of the hormone circulates bound. Also at this time the testosterone level is higher in boys than in girls. During these two periods, therefore, girls do not show high levels of testosterone. These fetal and neonatal peaks of testosterone, together with the functional steroid receptor activity, are, according to the current dogma, thought to fix the development of structures and circuits in the brain for the rest of a boy's life (producing "programming" or "organizing" effects). Later, the rising hormone levels that occur during puberty "activate" circuits and behavioral patterns that were built during development, in a masculinized and de-feminized direction for male brains or in a feminized and de-masculinized direction for female brains.

The brain structure differences that result from the interaction between hormones and developing brain cells are thought to be the major basis of sex differences in a wide spectrum of behaviors, such as gender role (behaving as a man or a woman in society), gender identity (the conviction of belonging to the male or female gender), sexual orientation (heterosexuality, homosexuality, or bisexuality), and sex differences regarding cognition, aggressive behavior, and language organization. Factors that interfere with the interactions between hormones and the developing brain systems during development in the womb may permanently influence later behavior.

As sexual differentiation of the genitals takes places much earlier in development (i.e., in the first two months of pregnancy) than sexual differentiation of the brain, which starts in the second half of pregnancy and becomes overt upon

reaching adulthood, these two processes may be influenced independently of each other. In rare cases, these two processes may be incongruent, providing one possible mechanism for transsexuality, that is, people with male sexual organs who feel female or vice versa. It also means that in the event of an ambiguous sex at birth, the degree of masculinization of the genitals may not always reflect the degree of masculinization of the brain (Hughes et al., 2006; Swaab, 2004, 2008). In addition, gender identity may be determined by prenatal hormonal influences, even though the prenatal hormonal milieu might be inadequate for full genital differentiation (Reiner, 1999).

Programmed gender identity is irreversible

The irreversibility of programmed gender identity is clearly illustrated by the sad story of the John–Joan–John case (i.e., the case of David Reimer). In the 1960s and 1970s, in the context of the concept of behaviorism, it was postulated that a child is born as a *tabula rasa* and is subsequently forced in the male or female direction by society's conventions. Although it is true that, in humans, self-face recognition appears to emerge at around 18 months of age (Keenan et al., 2000) and that by the age of 2–3 years children are able to correctly label themselves and others according to gender (Ahmed et al., 2004), there is no evidence that external or social events might modify these processes. However, J. Money argued that "Gender identity is sufficiently incompletely differentiated at birth as to permit successful assignment of a genetic male as a girl. Gender identity then differentiates in keeping with the experiences of rearing" (Money, 1975). This view had devastating results in the John–Joan–John case (Colapinto, 2001). Money maintained that gender imprinting does not start until the age of 1 year, and that its development is well advanced by the age of 3–4 years (Money and Erhardt, 1972). This was, indeed, the basis for the decision to make a girl out of an 8-month-old boy who lost his penis due to a mistake during minor surgery (i.e., an operation to correct phimosis). The testicles of this child were removed before he reached the age of 17 months in order to facilitate feminization. The child was dressed in girls' clothes, received psychological counseling, and was given estrogens in puberty. According to Money, this child developed as a normal female. However, Milton Diamond later made it clear that this had not been the case at all. In adulthood, this child changed back to male, married, and adopted several children (Diamond and Sigmundson, 1997). Unfortunately, he had a troubled life and committed suicide in 2004. This story illustrates the enormous programming influence of the intrauterine period on gender. Other cases have been described in the literature (Bradley et al., 1998), due to enzymatic disorders (al-Attia, 1996; Cohen-Kettenis, 2005; Praveen et al., 2008) or to cloacal exstrophy (Reiner, 2005), that support the existence of early permanent programming of brain sex by biological factors and androgen exposure, rather than by social environment and learning (Jürgensen et al., 2007; Swaab, 2004).

The mechanism of sexual differentiation of the brain: neurobiological factors

In male rats, testosterone is turned into estrogens by local aromatization in the brain, and these estrogens then masculinize certain brain areas. This finding agrees with the observation that, in partially androgen insensitive (testosterone feminized—Tfm) male rats, no reversion of the sex difference was present in the preoptic area (Gorski, 1984) and the bed nucleus of the stria terminalis (Garcia-Falgueras et al., 2005). These animals retained a male neuroanatomy. Other brain nuclei, such as the posteromedial amygdala, the ventromedial hypothalamus, and the locus coeruleus were, however, feminized in Tfm male rats (Morris et al., 2005; Zuloaga et al., 2008).

In humans, however, the main mechanism appears to involve a direct effect of testosterone

on the developing brain. Complete androgen insensitivity syndrome is caused by mutations in the receptor gene for androgens. Despite their genetic (XY) masculinity, affected individuals develop as phenotypical women and experience "heterosexual" sexual orientation, fantasies, and experiences, without gender problems (Wisniewski et al., 2000). On the other hand, when a male fetus has a 5α-reductase-2 or 17β-hydroxy-steroid-dehydrogenase-3 deficiency preventing peripheral testosterone from being transformed into dihydrotestosterone, a "girl" with a large clitoris is born. These children are generally raised as girls. However, when testosterone production increases in these XY children during puberty, this "clitoris" grows to penis size, the testicles descend, and the child's build begins to masculinize and become muscular. Despite the fact that these children are initially raised as girls, the majority (60%) change into heterosexual males (Cohen-Kettenis, 2005; Hughes et al., 2006; Imperato-McGinley et al., 1979; Praveen et al., 2008; Wilson et al., 1993), apparently due to the organizing effect of testosterone on early brain development. Boys who are born with a cloacal exstrophy—that is, with bladder exstrophy and a partly or wholly absent penis—are usually changed into girls immediately after birth. A survey showed that in adulthood only 65% of these children who were changed into girls continued to live as girls, and when individuals with gender dysphoria were excluded, the figure dropped to 47% (Meyer-Bahlburg, 2005; Reiner and Gearhart, 2004). From these examples, it appears that the direct action of testosterone on the developing brain in boys and the lack of it in the developing brain in girls are crucial factors in the development of male and female gender identity and sexual orientation, although other sexually dimorphic functions still need to be investigated in these people. Conversely, studies on cloacal exstrophy suggest that the postnatal testosterone peak is not crucial for gender identity development, given that these children generally undergo operation shortly after birth.

Recent data show that environmental compounds during early development may interfere with sexual differentiation of the human brain. Plastic softeners, that is, phthalate esters, are pervasive environmental chemicals with anti-androgenic effects. Exposure to these compounds is accompanied by reduced masculine play in boys (Swan et al., 2010). Higher prenatal polychlorinated biphenyls (PCB) levels were related with less masculine play in boys, while higher prenatal dioxin levels were associated with more feminized play in boys as well as in girls (Vreugdenhil et al., 2002). The effect of such environmental endocrine disruptors on sexual differentiation of brain systems should be further studied in future.

Sex differences in the human brain

A sex difference in brain weight is already present in children from the age of 2 years (Swaab and Hofman, 1984) and sex differences can thus be expected throughout the brain from early in development. In the adult human brain structural sex differences can be found from the macroscopic level (Goldstein et al., 2001) down to the ultramicroscopic level (Alonso-Nanclares et al., 2008). Functionally, too, a large number of sex differences in different brain regions have recently been described (Allen et al., 2003; Amunts et al., 1999, 2007; Savic, 2005; Savic and Lindstroom, 2008). Sexual differentiation of the human brain is also expressed in behavioral differences, including sexual orientation (homo-, bi-, and heterosexuality) and gender identity (Allen and Gorski, 1992; Hines, 2003; LeVay, 1991; Swaab, 2003), and in differences at the level of brain physiology and in the prevalence of neurological and psychiatric disorders (Bao and Swaab, 2007; Savic and Engel, 1998; Swaab, 2003). In the current review we focus on the sex differences in the human hypothalamus and adjacent areas.

When observed by Swaab's group, the structural difference in the intermediate nucleus of the human hypothalamus (InM) (Braak and

Braak, 1987; Brockhaus, 1942; Koutcherov et al., 2007) was found to be 2.5 times larger in men than in women and to contain 2.2 times as many cells (Swaab and Fliers, 1985). This InM nucleus was at first termed "the sexually dimorphic nucleus of the preoptic area (SDN-POA)" (Swaab and Fliers, 1985). In the preoptic area, Allen et al. (1989) described four interstitial nuclei of the anterior hypothalamus (INAH-1 to 4, while INAH-1 is identical to the InM/SDN-POA) and found a larger volume of the INAH-3 and INAH-2 subdivisions in men compared to women (respectively 2.8 and 2 times greater). The fact that they could not find a sex difference in INAH-1 (InM), as found by Swaab's group (Swaab and Fliers, 1985), could be fully explained by the strong age effect on the sex differences of this nucleus (Swaab, 2003; Swaab and Hofman, 1988). In fact, the sex difference develops only after the age of 5 years and disappears temporarily after the age of 50 years (Swaab and Fliers, 1985; Swaab et al., 1992). Further analysis of INAH-1 galanin cell population in the transsexual people and controls is ongoing and confirms the presence of a clear sex difference in adult controls up to 45 years of age.

The uncinate nucleus (Un) was localized and delineated using three different stainings, that is, thionin, neuropeptide-Y, and synaptophysin. We found sex differences in volume and neuron number in the INAH-3 subdivision while no differences were found for INAH-4 (Fig. 1; Garcia-Falgueras and Swaab, 2008). The presence of a sex difference in INAH-3 volume fully agreed with previously reported data (Allen et al., 1989; Byne et al., 2000, 2001; LeVay, 1991), as did the

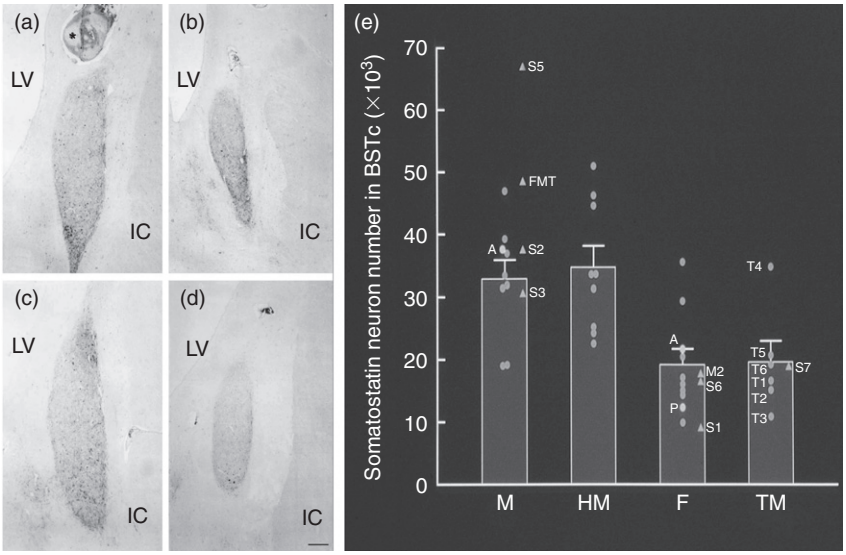

Fig.1. Representative immunocytochemical staining of the somatostatin neurons and fibers in the bed nucleus of the stria terminalis, central subdivision (BSTc) of a reference man (a), a reference woman (b), a homosexual man (c), and a male-to-female transsexual (d). *, Blood vessels; LV, lateral ventricle; IC, internal capsule. Bar represent 0.35 mm. (e) Graph of BSTc number of neurons in different groups according to sexual orientation and gender identity (M, heterosexual male reference group; HM, homosexual male group; F, female reference group; TM, male-to-female transsexual people; T1-T6, transsexual subjects; A, AIDS patient; P, postmenopausal woman; S7, Gender Identity Disorder subject). The sex hormone disorder patients S1, S2, S3, S5, S6, and M2 indicate that changes in sex hormone levels in adulthood do not change the neuron numbers of the BSTc. There is a statistical difference between the M and the TM group ($p < 0.04$) while no difference was between the heterosexual male reference group and the homosexual group. The female to male transsexual (FMT) subject is in the male range. From Kruijver et al. (2000) with permission.

sex difference for the number of neurons in INAH-3. A number of different names have been used to refer to the two Un subnuclei (Garcia-Falgueras and Swaab, 2008): (1) periventricular and uncinate nucleus (the former closer to the third ventricle than the latter) (Braak and Braak, 1987); (2) INAH-4 (closer to the third ventricle than the INAH-3) (Allen et al., 1989); and, most recently, (3) lateral and medial subdivisions of the Un (Koutcherov et al., 2007). In view of the evidence provided by neurochemical markers such as neuropeptide-Y and synaptophysin and the fact that they appear as one structure in some subjects, there are indeed arguments in favor of considering these two subdivisions a single structure called the Un. It has been suggested the INAH-3 was the homologue of the rat central nucleus of the medial preoptic area (Koutcherov et al., 2007) that, in this animal, is clearly related to the brain network for input and output of male sexual behavior (Schober and Pfaff, 2001; Swaab, 2004). On the other hand, the INAH-1 (InM) may be a candidate for that homology. Further research with specific markers is required to solve this issue.

Moreover, similar to the BSTc, the INAH-3 was found in male-to-female (MtF) transsexual people to be small (of female size and cell number), while the INAH-4 subdivision did not show gender-related differences, or any morphological sex difference between men and women (Fig. 1; Garcia-Falgueras and Swaab, 2008). Other sex differences have been found in the human anterior commissure, the interthalamic adhesion and in the corpora mammillaria (Allen and Gorski, 1991; Swaab, 2003).

Sex hormone receptors and neurosteroids

Sex hormone receptors, too, are expressed in a sexually dimorphic way in the human hypothalamus and adjacent areas.

In most hypothalamic areas that show androgen receptor staining, nuclear staining, in particular, is less intense in women than in men. The strongest sex difference was found in the lateral and the medial mammillary nucleus (MMN; Fernandez-Guasti et al., 2000). The mammillary body complex is known to be involved in several aspects of sexual behavior, such as arousal of sexual interest and penile erection (Fernandez-Guasti et al., 2000; MacLean and Ploog, 1962; Swaab, 2003). In addition, a sex difference in androgen receptor staining was present in the horizontal diagonal band of Broca, SDN-POA, medial preoptic area (mPOA), dorsal and ventral zone of the periventricular nucleus (PVN), supraoptic nucleus (SON), ventromedial hypothalamic nucleus, and infundibular nucleus (INF). However, no sex differences were observed in androgen receptor staining in the adult bed nucleus of the stria terminalis (BSTc), the nucleus basalis of Meynert, and the islands of Calleja (Fernandez-Guasti et al. 2000).

No differences related to male sexual orientation were found in nuclear androgen receptor activity in the mammillary complex, this activity not being found to differ in heterosexual men compared with homosexual men, but it was significantly stronger in men than in women. A female-like pattern was found in 26- and 53-year-old castrated men and in intact old men. These data indicate that the amount of nuclear receptor staining in the adult mammillary complex is dependent on the circulating levels of androgens rather than on gender identity or sexual orientation. This idea is supported by the findings that a male-like pattern of androgen receptor staining was found in a 36-years-old bisexual noncastrated MtF transsexual (T6) and a heterosexual virilized woman aged 46 (Kruijver et al., 2001), while a female-like pattern for INAH-3 volume and number of cells was found in the former patient (T6) (Garcia-Falgueras and Swaab, 2008).

Various sex differences have been observed for estrogen receptor α (ERα) staining in the hypothalamus and adjacent areas of young adult human subjects. More intense nuclear ERα immunoreactivity was found in young men compared with young women, for example, in the SDN-POA, the SON, and the PVN. Women showed a stronger nuclear ERα immunoreactivity in the suprachiasmatic nucleus (SCN) and MMN. No sex

differences in nuclear ERα staining were found in, for example, the bed nucleus of the stria terminalis (BSTc), the islands of Calleja (Cal), or the INF. More intense nuclear ERβ staining was found in men in, for example, the neurons of the BSTc, the islands of Calleja, and the InM/SDN-POA. Women showed more nuclear ERβ staining in the SCN, the SON, the PVN, the INF, and the MMN (Ishunina et al., 2007). Observations in subjects with abnormal hormone levels showed, in most areas, ERβ immunoreactivity distribution patterns that were consistent with the level of circulating estrogens, suggesting that the majority of the reported sex differences in ERβ immunoreactivity are "activational" rather than "organizational" in nature (Kruijver et al., 2002, 2003).

In the BSTc, differences in sex hormone receptors such as ERα, ERβ, androgen receptor (AR), and progesterone receptor (PR) are present from fetal age onward. More nuclear ERβ was observed in females than in males during the fetal/neonatal ages, whereas there were no overt sex differences in the other three sex hormone receptors detected. In adult men, ERα and PR immunoreactivity was more pronounced in the BSTc of men than in women (Chung, 2003). Hence, the sensitivity of the BSTc for the different sex hormones depends strongly on sex and age.

Transsexuality

There is a vast array of factors that may lead to gender problems (Table 1). Twin and family research has shown that genetic factors play a part (Coolidge et al., 2002; Gómez-Gil et al., 2010a; Hare et al., 2009; van Beijsterveldt et al., 2006). Rare chromosomal abnormalities may lead to transsexuality (Hengstschläger et al., 2003) and it was found that polymorphisms of the genes for ERα and ERβ, AR repeat length polymorphism and polymorphisms in the aromatase or CYP17 gene also produced an increased risk (Bentz et al., 2008; Hare et al., 2009; Henningsson et al., 2005).

Abnormal hormone levels during early development may play a role, as suggested by the high frequency of polycystic ovaries, oligomenorrhea and amenorrhea in female-to-male (FtM) transsexuals. This observation suggests early intrauterine exposure of the female fetus to abnormally

Table 1. Prenatal factors that influence gender identity (the conviction of being a man or a woman) and that may result in transsexuality

Genetic factors	Rare chromosomal disorders (Hengstschläger et al., 2003)
	Twin studies (van Beijsterveldt et al., 2006; Coolidge et al., 2002; Gómez-Gil et al., 2010a; Hare et al., 2009)
	Polymorphisms in ERβ, androgen receptor, and aromatase genes (Bentz et al., 2008; Hare et al., 2009; Henningsson et al., 2005)
Hormones	Phenobarbital/diphantoin taken by pregnant mother (Dessens et al., 1999)
	Hormones, cloacal exstrophy (Meyer-Bahlburg, 2005; Reiner and Gearhart, 2004)
	5α-reductase-2 or 17β-hydroxy-steroid-dehydrogenase-3 deficiency (Cohen-Kettenis, 2005; Hughes et al., 2006; Imperato-McGinley et al., 1979; Praveen et al., 2008; Wilson et al., 1993)
	Girls with CAH (Dessens et al., 2005; Meyer-Bahlburg et al., 1995, 1996; Zucker et al., 1996)
	Complete androgen insensitivity syndrome results in XY heterosexual females with feminine identity (Wisniewski et al., 2000)
	DES sons: 25% gender problems (http://des-sons.grouply.com/login/)
Immune response	Fraternal birth order (Gómez-Gil et al., 2010b)
Social factors	Postnatally no evidence (Cohen-Kettenis and Gooren, 1999; Colapinto, 2001; Diamond and Sigmundson, 1997; Swaab, 2004)

Abbreviations: CAH, congenital adrenal hyperplasia; DES, diethylstilbestrol.

high levels of testosterone (Padmanabhan et al., 2005). A recent study did not confirm a significantly increased prevalence of polycystic ovary syndrome. However, there was a significantly higher prevalence of hyperandrogynism in FtM transsexuals, also indicating the possible involvement of high testosterone levels in transsexuality (Mueller et al., 2008). A girl with congenital adrenal hyperplasia (CAH), who has been exposed to extreme levels of testosterone in utero, will also have an increased chance of becoming transsexual. Although the likelihood of transsexuality developing in such cases is 300–1000 higher than normal, the risk for transsexuality in CAH is still only 1–3% (Zucker et al., 1996), whereas the probability of serious gender problems is 5.2% (Dessens et al., 2005). The consensus is, therefore, that girls with CAH should be raised as girls, even when they are masculinized (Hughes et al., 2006).

Epileptic women who were given phenobarbital or diphantoin during pregnancy also have an increased risk of giving birth to a transsexual child. Both these substances change the metabolism of the sex hormones and can act on the sexual differentiation of the child's brain. In a group of 243 women who had been exposed to such substances during pregnancy, Dessens et al. (1999) found three transsexual children and a few others with less radical gender problems; these are relatively high rates for such a rare condition. On the "DES" (diethylstilbestrol, an estrogen-like substance—see later) children's website they claimed that transsexuality occurs in 35.5% and a gender problem in 14% of the DES cases (links GIRES and DES SONS webpages). This is alarming, but needs, of course, to be confirmed in a formal study. There are no indications that postnatal social factors could be responsible for the occurrence of transsexuality (Cohen-Kettenis et al., 1998).

In addition, homosexual MtF transsexual people were found to have a later birth order and more brothers than sisters (Gómez-Gil et al., 2010b), suggesting the presence of immunological processes during pregnancy directed toward products of the Y chromosome.

It should be noted that only in 23% of cases does a childhood gender problem lead to transsexuality in adulthood. With regard to sexual orientation, the most likely outcome of childhood gender identity disorder is homosexuality or bisexuality (Cohen-Kettenis and Gooren, 1999; Coolidge et al., 2002; Wallien and Cohen-Kettenis, 2008). Moreover for the diagnosis of transsexuality other disorders inducing temporal transsexual desires—such as bipolar psychosis, schizophrenia, and personality disorders—should be excluded (à Campo et al. 2003; Habermeyer et al., 2003; Mouaffak et al., 2007).

Transsexuality and the brain

The theory on the origins of transsexuality is based on the fact that the differentiation of sexual organs takes place during the first couple of months of pregnancy, before the sexual differentiation of the brain. As these two processes have different timetables, it is possible, in principle, that they take different routes under the influence of different factors. If this is the case, one might expect to find, in transsexuals, female structures in a male brain and vice versa, and indeed, we did find such reversals in the central nucleus of the BSTc and in the INAH-3 (Figs. 1 and 2), two brain structures that, in rats, are involved in many aspects of sexual behavior. However, a gender identity test for rats does not exist, and this hypothesis can therefore be studied only in humans.

We found a clear sex difference in the human BSTc and INAH-3. In men, the BSTc area was twice that found in women and contained twice as many somatostatin neurons (Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995). The same was true of the INAH-3, which was found to be 1.9 times larger in men than in women and to contain 2.3 as many neurons (Fig. 2; Garcia-Falgueras and Swaab, 2008). In relation to sexual orientation, no difference was found in the size or number of neurons in the BSTc area, while for the INAH-3 the volume has previously been found to be related to sexual

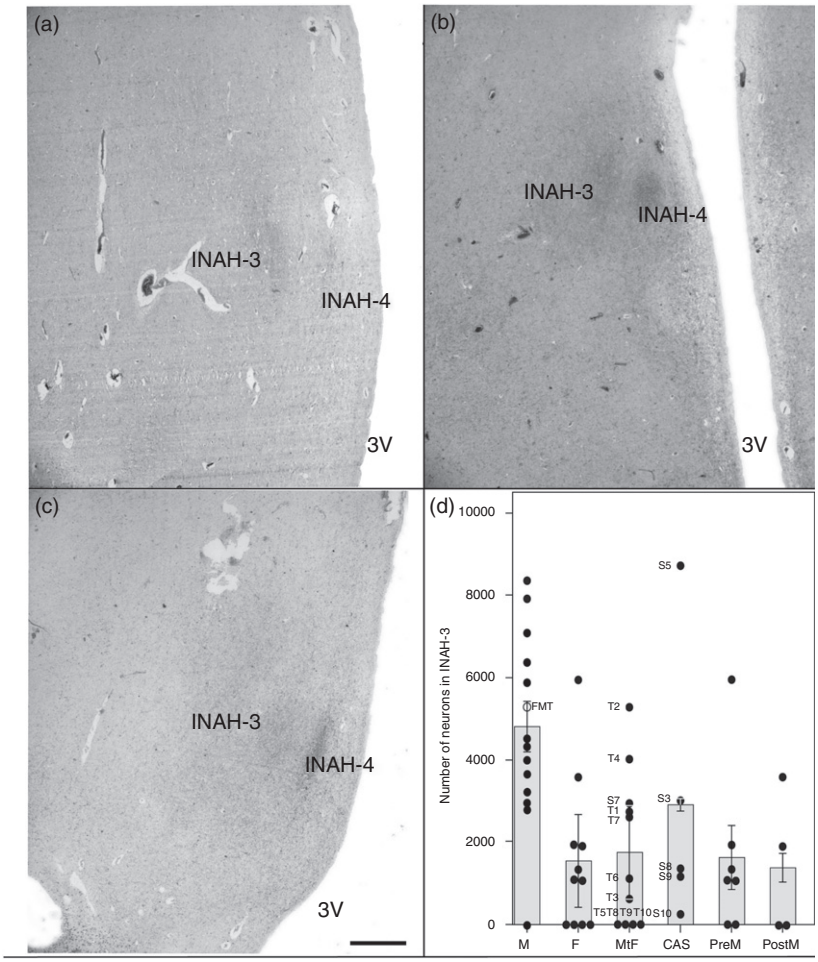

Fig. 2. Representative immunocytochemical staining of the NPY innervation of the uncinate nucleus (INAH-3 and INAH-4) of a reference man (a), a reference woman (b), and a male-to-female transsexual (c). Note that the size is larger in the male group (a) than in the other two groups (b and c). Bar represent 500 mm. (d) Distribution of the INAH-3 number of neurons among different groups according to their gender identity and hormonal changes in adulthood. M, control male group; F, control female group; MtF, male-to-female transsexual group; CAS, castrated male group; PreM, premenopausal women; PostM, postmenopausal women T1-T10, transsexual subjects; S3, S5, S8, S9, S10, castrated subjects because of prostate cancer. Bars represent means and standard errors of the mean. Statistically significant differences were found between men (M) and women (F) ($p < 0.029$) and between men (M) and male-to-female transsexual MtF groups ($p < 0.002$). The female to male transsexual subject (FTM), in the male group, had a masculine INAH-3 number of neurons and the untreated S7 subject, in the MtF group, had a similar number of neurons to the other transsexuals examined. (a, b, c and d) Adapted from Garcia-Falgueras and Swaab (2008) with permission.

orientation, being larger in heterosexual than in homosexual men (Byne et al., 2001; LeVay, 1991). In MtF transsexuals, we found a completely female BSTc and INAH-3. Until now, we have only been able to obtain material from one FtM transsexual, and his BSTc and INAH-3 indeed turned out to have all the male characteristics. We were able to exclude the possibility that the reversal of sex differences in the BSTc and INAH-3 were caused by changing hormone levels in adulthood

(Garcia-Falgueras and Swaab, 2008; Kruijver et al., 2000; Zhou et al., 1995), and it therefore seems that we are dealing with a developmental effect. Our observations thus support the above-mentioned neurobiological theory about the origin of transsexuality. The size of the BSTc and the INAH-3 and their number of neurons match the gender that transsexual people feel they belong to, and not the sex of their sexual organs, birth certificate or passport. Unfortunately, the sex difference in the BSTc volume does not become apparent until early adulthood (Chung et al., 2002), meaning that this nucleus cannot be used for early diagnosis of transsexualism.

One person we studied had untreated male gender dysphoria (S7), took no hormones and kept his transsexual feelings under wraps. He appeared to have a large INAH-3 volume—in the male range—but a female INAH-3 number of neurons (Garcia-Falgueras and Swaab, 2008; Fig. 2d) and a female BSTc somatostatin neuron number (Kruijver et al., 2000). Hence, this individual's hypothalamic characteristics were mid-way between male and female values.

In transsexual MtF patients who receive hormonal treatment, some intermediate values, between those typical for men and women, have been found for lateralization and cognitive performance (Cohen-Kettenis et al., 1998). Recently, functional reversals have been reported in the brains of transsexual people. A PET study in non-homosexual MtF transsexual people (i.e., erotically attracted to women), who were not treated hormonally, showed that a number of brain areas in the transsexual hypothalamus were activated by pheromones in a sex-atypical way. Although the functional reactions in the hypothalamus to an estrogen-derived pheromone were predominantly female, MtF transsexual people also showed some characteristics of a male activation pattern (Berglund et al., 2008). Also studies of mental rotation task, in which men typically outperform women, showed an "in-between" pattern in MtF transsexuals. Compared to control males, the activation in MtF transsexuals during the task was, like in female controls, lower in the superior parietal lobe. MtF transsexuals differed, however, also from the females, and showed higher activation in orbital and right dorsolateral prefrontal regions and lower activation in the left prefrontal gyrus. Interestingly, the reduced parietal activation in MtF transsexuals was correlated with years of estrogen treatment (Carrillo et al., 2010), suggesting that a major reason for the observed "female feature" could have been the hormone supplement treatment. When viewing erotic stimuli, MtF transsexuals before treatment tended to display female-like cerebral processing on functional magnetic resonance imaging (fMRI). The core network consisting of the occipitotemporal cortex, anterior cingulate cortex, medial prefrontal cortex, pre- and postcentral cortex, thalamus, hypothalamus, and bilateral amygdala was activated in males, females, and MtF transsexuals. The three latter regions, however, were more activated in male controls than in female controls and MtF transsexuals (Gizewski et al., 2009). One possible explanation could be that both females and MtF transsexuals reported a lower degree of sexual arousal, and particularly the hypothalamus activation is reported to arousal-dependent. Transsexual persons have recently been investigated with diffusion tensor imaging (DTI), which measures fractional anisotropy (FA) and provides information about neuronal fiber tracts. The study showed significantly higher FA values in the medial and posterior parts of the right superior longitudinal fasciculus (SLF), the forceps minor, and the corticospinal tract in male controls and FtM transsexuals compared to control females (Rametti et al., 2010). In contrast to these two studies, which suggested sex atypical parietal activations and fronto-parietal neuronal connections, no difference from sex matched controls were detected in a comparative study of regional gray and white matter volumes, with exception for an increase in gray matter volume in the left putamen in MtF transsexuals compared to both male and female controls (Luders et al., 2009). Recently, Savic and coworkers combined voxel-based

morphometry and structural volumetry to find that MtF transsexuals have reduced structural volumes of the putamen and thalamus compared to both male and female controls. In addition, their gray matter fraction in the right insular cortex, and the right temporo-parietal junction was larger than in both control groups. Together, these anatomical findings question the dogma that transsexual persons simply have an inverted sex dimorphism of the brain in relation to their biological sex. The findings also raise question as to whether transsexuality may be associated with changes in the cerebral networks involved in self-perception—the temporo-parietal junction, the thalamus, and the insular-inferior frontal cortex (Northoff et al., 2006).

Sexual orientation: heterosexuality, homosexuality, and bisexuality

Sexual orientation in humans is also determined during early development, under the influence of our genetic background and factors that influence the interactions between the sex hormones and the developing brain (Table 2).

The apparent impossibility of getting someone to change their sexual orientation is a major argument against the importance of the social environment in the emergence of homosexuality, as well as against the idea that homosexuality is a lifestyle choice. The mind boggles at the methods used in the attempt to bring about changes in sexual orientation: hormonal treatments such as castration, administration of testosterone or estrogens (treatments that appeared to affect libido but not sexual orientation); psychoanalysis; apomorphine administered as an emetic in combination with homoerotic pictures; psychosurgery (lesions in the hypothalamus); electroshock treatment; chemical induction of epileptic insults and imprisonment. As none of these interventions has led to a well-documented change in sexual orientation (LeVay, 1996), there can be little doubt that our sexual orientation is fixed by the time we reach adulthood and is beyond further influence. Changes in sexual orientation in adulthood have been described—for example, from heterosexual to pedophile—but only in cases of brain tumors in the hypothalamus and prefrontal cortex (Burns and Swerdlow, 2003; Miller et al., 1986). However, these devastating changes in the hypothalamus are too large to interpret them in terms of functional changes in particular neuronal circuits. There are also claims that pedophiles and homosexual men have switched to heterosexual behavior as a result of stereotactical psychosurgery (lesions in the nucleus ventromedialis) (Dieckmann and Hassler, 1977), but these interventions are not only ethically questionable, they also do not meet any

Table 2. Prenatal factors that may influence sexual orientation (homosexuality, heterosexuality, bisexuality)

Genetic factors	Twin studies (Bailey and Bell, 1993; Bockalandt and Vilain, 2007; LeVay and Hamer, 1994) Molecular genetics (Swaab, 2004)
Hormones	Girls with CAH (Meyer-Bahlburg et al., 1995, 1996; Swaab, 2004; Zucker et al., 1996) DES (Cohen-Kettenis et al., 1998; Ehrhardt et al., 1985; Swaab, 2004)
Chemical factors	Prenatal exposure to nicotine, amphetamines, or thyroid medication (Ellis and Cole-Hardin, 2001; Ellis and Hellberg, 2005)
Immune response?	Homosexual orientation in men is most likely to occur in men with a large number of older brothers (Blanchard, 2001; Bogaert, 2003)
Social factors?	Stress in the mother during pregnancy (Bailey et al., 1991; Bogaert, 2003; Ellis et al., 1988) Being raised by transsexual or homosexual parents does not affect sexual orientation (Green, 1978)

Abbreviations: CAH, congenital adrenal hyperplasia; DES, diethylstilbestrol.

scientific standards. There are also some recent reports postulating that the sexual orientation of homosexual women, more than that of homosexual men, may sometimes change, either spontaneously or under the influence of psychotherapy (Spitzer, 2003). The effectiveness of therapy and the absence of bisexuality has, however, never been convincingly demonstrated in these cases.

The presence of a substantial genetic component in the development of sexual orientation is apparent from family and twin studies (Bailey and Bell, 1993; Bocklandt and Vilain, 2007). However, exactly which genes play a role is not yet clear. According to LeVay and Hamer (1994), the size of the genetic component in homosexuality for both sexes is over 50%. A number of genetic studies have suggested maternal transmission, indicating X-linked inheritance. The X chromosome has accumulated genes involved in sex, reproduction, and cognition. A meta-analysis of four linkage studies suggested that Xq28 plays an important role in male homosexuality (Hamer et al., 1993). However, 16 years after the initial findings the exact genes involved have not yet been identified (Bocklandt and Vilain, 2007). A different technique also indicated a role for the X chromosome in male sexual orientation. Women with gay sons appeared to have an extreme skewing of X-inactivation when they are compared to mothers without gay sons (Bocklandt et al., 2006). Although this unusual methylation pattern supports a possible role of the X chromosome in male homosexuality, its mechanism of action is far from clear. Given the complexity of the development of sexual orientation, it is likely to involve many genes. A genome-wide linkage screening indeed identified several chromosomal regions and candidate genes for further exploration (Mustanski et al., 2005).

Whatever the exact nature of the genetic factor, it is interesting that such a factor has stayed present in the population throughout human history, given that homosexuals do not tend to procreate as much as the rest of the population. A good explanation could be that the genetic factors that are responsible for homosexuality also have a beneficial effect on the procreation of the population. Indeed, Camperio Ciani et al. (2004) have found that women on a homosexual male's mother's side tend to be more fertile. This antagonistic inheritance that promotes fecundity in females and a homosexual orientation in males is partly linked to the X chromosome (Iemmola and Camperio Ciani, 2009).

Abnormal hormone levels originating from the child itself during intrauterine development may influence sexual orientation, as is apparent from the large percentage of bisexual and homosexual girls with CAH (Meyer-Bahlburg et al., 1995, 1996; Zucker et al., 1996). Between 1939 and 1960, some two million pregnant women in the United States and Europe were prescribed diethylstilbestrol (DES) in order to prevent miscarriage. DES is an estrogen-like substance that actually turned out not to prevent miscarriage; furthermore, it also found, in small dosages, not only to give a slightly elevated risk of cervical cancer but also to increase the chance of bisexuality or lesbianism in adult woman (Ehrhardt et al., 1985; Meyer-Bahlburg et al., 1996; Titus-Ernstoff et al., 2003) although this was not confirmed in an other study (Ellis et al., 1988).

The chance that a boy will be homosexual increases with the number of older brothers he has. This phenomenon is known as the fraternal birth order effect and is putatively explained by an immunological response by the mother to a product of the Y chromosome of her sons. The chance of such an immune response to male factors would increase with every pregnancy resulting in the birth of a son (Blanchard, 2001; Bogaert, 2003). Prenatal exposure to nicotine, amphetamine, or thyroid-gland hormones increases the chances of giving birth to lesbian daughters (Ellis and Cole-Harding, 2001; Ellis and Hellberg, 2005). A stressed pregnant woman has a greater chance of giving birth to a homosexual son (Ellis and Cole-Harding, 2001; Ellis et al., 1988) or a lesbian daughter (Bailey et al., 1991) (Table 2).

Although it has often been postulated that postnatal development is also important for the

direction of sexual orientation, there is no solid proof for this. On the contrary, children who were born after artificial insemination with donor sperm and who were raised by a lesbian couple are heterosexually oriented (Green, 1978). There is also no proof for the idea that homosexuality is the result of a deficient upbringing, or that it is a "lifestyle choice" or an effect of social learning (LeVay, 1996). It is curious, therefore, that some children are still forbidden to play with homosexual friends, an unthinkable attitude left over from the idea that homosexuality is "contagious" or can be learned.

Sexual orientation and the brain

Clinical observations have shown the involvement of a number of brain structures in sexual orientation. It has been reported that in some patients with Klüver-Bucy syndrome, which involves lesions of the temporal lobe, orientation changed from heterosexual to homosexual. Shifts in sexual orientation (to homosexual and pedophile) have also been reported in connection with tumors in the temporal lobe and hypothalamus. Lesions in the preoptic area of the hypothalamus in male rodents, such as ferrets and rats, produce shifts in sexual orientation (Swaab, 2003). Lesions of the same structure in their female conspecifics do not change sexual behavior. Instead, female rats become aggressive toward male intruders and start approaching their female conspecifics upon lesion of the ventromedial hypothalamic nuclei (Kindon et al., 1996; Leedy, 1984; Paredes and Baum, 1995).

Of interest is also that male rat knockouts lacking Ca-TRP channels (TRPC2), which are necessary for pheromone signal transduction, do not approach to fertile females, but do mount male rats (Zufall, 2005). These data have two implications: first, intact pheromone signal detection, as well as an intact hypothalamic transduction seems necessary for heterosexual behavior. Second, the hypothalamic nuclei mediating sexual behavior seem, at least in some rodents, to differ between males and females. The exact function of the these nuclei is not well known, but it seems to be crucial for the approach to a sexual partner, since it is implicated in the recognition and integration of sensory stimuli such as sexual clues, in arousal mechanisms and in copulatory behavior and its motor expression (Schober and Pfaff, 2007; Swaab, 2003).

Several structural and functional differences in the brain have been described in relation to sexual orientation (for a review see Swaab, 2008). Swaab's group found the first difference in the SCN, or brain clock, which turned out to be twice as large in homosexual compared with heterosexual men (Swaab and Hofman, 1990). In an experiment with rats a similar difference could be induced, by pharmacologically disturbing the interaction between testosterone and the developing brain around the time of birth, using the aromatase inhibitor 1,4,6-androstatrien-3,17-dione (ATD) in the neonatal period. This experiment yielded bisexual adult rats, which had larger numbers of cells in their SCN (Swaab et al., 1995). The difference in the SCN was therefore not caused by a change in sexual behavior, as was suggested at the time, but by a disturbed interaction between sex hormones and the developing brain. In 1991, LeVay reported that homosexual men, just like heterosexual women, have a smaller volume of hypothalamic nucleus (INAH-3) (LeVay, 1991). No differences were found in the BSTc volume or number of somatostatine neurons in homosexual men compared to heterosexual men (Kruijver et al., 2000; Zhou et al., 1995). In 1992, Allen and Gorski reported that the anterior commissure of homosexual men is larger than that of heterosexual men (Allen and Gorski, 1992). This structure, which is larger in women than in men, takes care of left–right connections within the temporal cortex and is thus involved in sex differences in cognitive abilities and language. The difference in its size may possibly be related to the sex-atypical hemispheric asymmetries observed in homosexual men and homosexual women by Savic and Lindström (2008). Witelson et al. (2008) recently reported that the isthmal

area of corpus callosum was larger in the homosexual compared to heterosexual men, which also could contribute to the observed differences in hemispheric asymmetry.

Emerging studies with functional imaging show differences in the hypothalamus activation in relation to sexual orientation. The first brain imaging paper to point out differences in the hypothalamus in relation to sexual orientation by means of fluorodeoxy glucose (FDG)—PET, by Kinnunen et al. (2004), did not receive much scientific or public attention, although it may have clinical consequences. The hypothalamus of homosexual men turned out not to be as responsive to a classic antidepressant (fluoxetine) as that of heterosexual men, which suggests a different kind of activity of the serotonergic system. Savic et al. (2001) used androstadienone, a pheromone-like compound derived from progesterone and excreted in perspiration in concentrations. Smelling of this compound activated the hypothalamus of heterosexual women and homosexual men in the same way, but did not elicit any hypothalamus response in heterosexual men. Apparently in heterosexual men the hypothalamic pathway is not stimulated by a male body-scent, which suggests that pheromone-like compounds in humans may contribute to determining our behavior in relation to our sexual orientation (Savic et al., 2005). In a follow-up study (Berglund et al., 2006), lesbian women, as compared to heterosexual women, reacted in a sex-atypical, almost reciprocal way (Fig. 3). These observations, too, show that there are hypothalamic circuits that function in a way that depends on our sexual orientation. The hypothalamic circuits are incorporated in the core network system for sexual arousal (Karama et al., 2002). Interestingly, when balancing for the degree of sexual arousal, this network seems similar in homo- and heterosexual subjects. Just like the pheromone responding core network, the triggering stimulus is reciprocal in homosexual compared to heterosexual subjects. Indeed, viewing erotic videos of heterosexual or homosexual content produced activation in the hypothalamus, detectable by fMRI, but only when subjects were

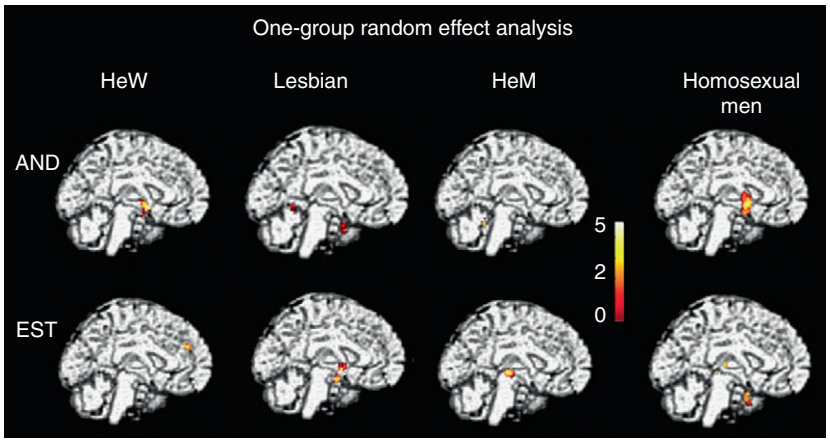

Fig. 3. Illustration of group-specific activations with the two putative pheromones (AND and EST). AND, androstadienone. EST, estratetraenol, is derivative of estrogene. The Sokoloff color scale illustrates *Z*-values reflecting the degree of activation (0.0–5.0). Because the same brain section is chosen, the figures do not always illustrate maximal activation for each condition (*Upper*). Cerebral activation during smelling of AND and EST. Clusters of activated regions are superimposed on the standard MRI brain (midsagittal plane). HeW, heterosexual women; HeM, heterosexual men. Note that there are hypothalamic circuits that function in a way that depends on our sexual orientation. From Berglund et al. (2006) with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this book.)

viewing videos of their respective sexual orientation (Paul et al., 2008). Accordingly Ponseti et al. (2006, 2009) found that neuronal response of the ventral striatum and the centromedian thalamus was stronger to prefer relative to non-preferred stimuli. Using fMRI, Kranz and Ishai found that face perception is modulated by sexual preference. Looking at a female face made the thalamus and medial prefrontal cortex of heterosexual men and homosexual women react more strongly, whereas in homosexual men and heterosexual women these structures reacted more strongly to the face of a man (Kranz and Ishai, 2006). A sexual-orientation-related difference in processing neuronal networks was suggested only by Hu et al. (2008). However, their subjects viewed erotic film involving mixed and same sex couples, evoking different levels of sexual arousal and disgust in homo- and heterosexual subjects, which may account for the detected differences. While being compelling in pinpointing the neuronal circuits for sexual attraction and arousal, these data cannot explain why the object of arousal differs.

Savic's previous studies raised the question of whether certain sexually dimorphic features in the brain, which are unlikely to be directly involved in reproduction, may differ between homosexual and heterosexual individuals. This issue was explored by studying hemispheric asymmetry, using volumetric MRI, and functional connectivity of the amygdala, using PET measurements of cerebral blood flow (Savic and Lindström, 2008). Volumetric measurements in heterosexual men and homosexual women showed a rightward cerebral asymmetry, whereas the volumes of the cerebral hemispheres were symmetrical in homosexual men and heterosexual women (Savic and Lindström, 2008). Moreover, homosexual subjects also showed sex-atypical amygdala connections. In homosexual men, as in heterosexual women, the connections were more widespread from the left amygdala. In homosexual women and heterosexual men, on the other hand, they were more widespread from the right amygdala. Furthermore, in homosexual men and heterosexual women the connections displayed were primarily with the contralateral amygdala and the anterior cingulate, while in heterosexual men and homosexual women the connections displayed were primarily displayed with the caudate, putamen, and the prefrontal cortex (Savic and Lindström, 2008). In verbal fluency and other verbal skills a lesbian group presented different values from the other three groups (heterosexual woman, heterosexual man, and homosexual man) (Rahman et al., 2003). Moreover dichotic listening performance has also been found to show a greater right ear advantage in heterosexual men as compared to heterosexual women, while lesbian women were somewhat masculinized in their functional cerebral asymmetry (Rahman and Koerting, 2008). Interestingly, lesbian women were recently found to have less gray matter bilaterally in the temporo-basal cortex, ventral cerebellum, and left ventral premotor cortex in relation to heterosexual women (Ponseti et al., 2009).

Together, these later studies suggest a linkage between sexual orientation and neurobiological entities that cannot be primarily linked to reproduction.

Conclusions

The human fetal brain becomes sex differentiated through direct hormone-independent effects of X and Y chromosome genes or through different levels of gonadal hormones during both prenatal and postnatal periods. The latter pathway is more powerful. By a direct action of testosterone the fetal brain develops into the male direction, and in absence of this hormone into the female direction. During the intrauterine period, gender identity (the conviction of belonging to the male or female gender), sexual orientation, cognition, aggression, and other behaviors are programmed in the brain in a sexually differentiated way. Sexual differentiation of the genitals takes place in the first two months of pregnancy, whereas sexual differentiation of the brain starts in the second half of

pregnancy. This means that in the event of an ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain.

Our observations on reversed sex differences in the brains of transsexual people support the idea that transsexuality, at least to some extent, is based on an opposite sexual differentiation of (1) sexual organs during the first couple of months of pregnancy and (2) the brain in the second half of pregnancy. There is no proof that the social environment after birth has an effect on the development of gender or sexual orientation, while the possible effects on sexual differentiation of the brain by endocrine disrupters in the environment and in medicines given to the pregnant mother should be investigated.

The differences observed in the INAH-3 in relation to sexual orientation and gender identity and this structure's possible connection with the BSTc suggest that these two nuclei and the two earlier described nuclei that were found to be related to gender and sexual orientation, that is, the SDN-POA (= InM = INAH-1) and SCN, are all part of a complex network involved in various aspects of sexual behavior. Neurobiological research on sexual orientation and gender identity in humans is only just gathering momentum, but the evidence shows that humans have a vast array of brain differences, related not only to gender, but also to sexual orientation. There is a need for further multidisciplinary research on the putative influence of testosterone in development, for example, in individuals with complete androgen insensitivity syndrome.

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Reference

à Campo, J., Nijman, H., Merckelbach, H., & Evers, C. (2003). Psychiatric comorbidity of gender identity disorders: a survey among dutch psychiatrists. *The American Journal of Psychiatry*, *160*, 1332–1336.

Ahmed, S. F., Morrison, S., & Hughes, I. A. (2004). Intersex and gender assignment; the third way? *Archives of Disease in Childhood*, *89*, 847–850.

al-Attia, H. M.(1996). Gender identity and role in a pedigree of arabs with intersex Due To 5 alpha reductase-2 deficiency. *Psychoneuroendocrinology*, *21*, 651–657.

Allen, J. S., Damasio, H., Grabowski, T. J., Bruss, J., & Zhang, W. (2003). Sexual dimorphism and asymmetries in the gray-white composition of the human cerebrum. *NeuroImage*, *18*, 880–894.

Allen, L. S., & Gorski, R. A. (1991). Sexual dimorphism of the anterior commissure and massa intermedia of the human brain. *Journal of Comparative Neurology*, *312*, 97–104.

Allen, L. S., & Gorski, R. A. (1992). Sexual orientation and the size of the anterior commissure in the human brain. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 7199–7202.

Allen, L. S., Hines, M., Shryne, J. E., & Gorski, R. A. (1989). Two sexually dimorphic cell groups in the human brain. *Journal of Neuroscience*, *9*, 497–506.

Alonso-Nanclares, L., Gonzalez-Soriano, J., Rodriguez, J. R., & DeFelipe, J. (2008). Gender differences in human cortical synaptic density. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 14615–14619.

Amunts, K., Schleicher, A., Bürgel, U., Mohlberg, H., Uylings, H. B., & Zilles, K. (1999). Broca's region revisited: Cytoarchitecture and intersubject variability. *Journal of Comparative Neurology*, *412*, 319–341.

Amunts, K., Schleicher, A., & Zilles, K. (2007). Cytoarchitecture of the cerebral cortex—more than localization. *NeuroImage*, *37*, 1061–1065.

Bailey, J. M., & Bell, A. P. (1993). Familiality of female and male homosexuality. *Behavior Genetics*, *23*, 313–322.

Bailey, J. M., Willerman, L., & Parks, C. (1991). A test of the maternal stress theory of human male homosexuality. *Archives of Sexual Behavior*, *20*, 277–293.

Bakker, J., & Baum, M. J. (2008). Role for estradiol in female-typical brain and behavioral sexual differentiation. *Frontiers in Neuroendocrinology*, *29*, 1–16.

Bakker, J., De Mees, C., Douhard, Q., et al. (2006). Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. *Nature Neuroscience*, *9*, 220–226.

Bao, A. M., & Swaab, D. F. (2007). Gender difference in age-related number of corticotropin-releasing hormone-expressing neurons in the human hypothalamic paraventricular nucleus and the role of sex hormones. *Neuroendocrinology*, *85*, 27–36.

Bentz, E. K., Hefler, L. A., Kaufmann, U., Huber, J. C., Kolbus, A., & Tempfer, C. B. (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*, 56–59.

Berglund, H., Lindström, P., Dhejne-Helmy, C., & Savic, I. (2008). Male-to-female transsexuals show sex-atypical hypothalamus activation when smelling odorous steroids. *Cerebral Cortex*, *18*, 1900–1908.

Berglund, H., Lindström, P., & Savic, I. (2006). Brain response to putative pheromones in lesbian women. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 8269–8274.

Blanchard, R. (2001). Fraternal birth order and the maternal immune hypothesis of male homosexuality. *Hormones and Behavior*, *40*, 105–114.

Bocklandt, S., Horvath, S., Vilain, E., & Hamer, D. H. (2006). Extreme skewing of X chromosome inactivation in mothers of homosexual men. *Human Genetics*, *118*, 691–694.

Bocklandt, S., & Vilain, E. (2007). Sex differences in brain and behavior: Hormones versus genes. *Advances in Genetics*, *59*, 245–266.

Bogaert, A. F. (2003). The interaction of fraternal birth order and body size in male sexual orientation. *Behavioral Neuroscience*, *117*, 381–384.

Braak, H., & Braak, E. (1987). The hypothalamus of the human adult: Chiasmatic region. *Anatomy and Embryology*, *175*, 315–330.

Bradley, S. J., Oliver, G. D., Chernick, A. B., & Zucker, K. J. (1998). Experiment of nurture: Ablatio penis at 2 months, sex reassignment at 7 months, and a psychosexual follow-up in young adulthood. *Pediatrics*, *102*, e9.

Brockhaus, H. (1942). Beitrag zur normalen anatomie des hypothalamus und der zona incerta beim menschen. *Journal of Psychology Neurology*, *51*, 96–196.

Burns, J. M., & Swerdlow, R. H. (2003). Right orbitofrontal tumor with pedophilia symptom and constructional apraxia sign. *Archives of Neurology*, *60*, 437–440.

Byne, W., Lasco, M. S., Kemether, E., et al. (2000). The interstitial nuclei of the human anterior hypothalamus: An investigation of sexual variation in volume and cell size, number and density. *Brain Research*, *856*, 254–258.

Byne, W., Tobet, S., Mattiace, L. A., et al. (2001). The interstitial nuclei of the human anterior hypothalamus: An investigation of variation with sex, sexual orientation, and HIV status. *Hormones and Behavior*, *40*, 86–92.

Camperio Ciani, A., Corna, F., & Capiluppi, C. (2004). Evidence for maternally inherited factors favouring male homosexuality and promoting female fecundity. *Proceedings Biological Sciences*, *271*, 2217–2221.

Carrillo, B., Gómez-Gil, E., Rametti, G., Junque, C., Gomez, A., Karadi, K., et al. (2010). Cortical activation during mental rotation in male-to-female and female-to-male transsexuals under hormonal treatment. *Psychoneuroendocrinology*, *35*, 1213–1222.

Chung, W.Ch. J.. (2003). Doctoral Thesis: Sexual differentiation of the human and rodent forebrain: gonadal steroids receptors and apoptosis in the bed nucleus of the stria terminalis and medial preoptic nucleus. January 15th 2003.

Chung, W. C., De Vries, G. J., & Swaab, D. F. (2002). Sexual differentiation of the bed nucleus of the stria terminalis in humans may extend into adulthood. *Journal of Neuroscience*, *22*, 1027–1033.

Cohen-Kettenis, P. T. (2005). Gender change in 46,XY persons with 5alpha-reductase-2 deficiency and 17beta-hydroxysteroid dehydrogenase-3 deficiency. *Archives of Sexual Behavior*, *34*, 399–410.

Cohen-Kettenis, P. T., & Gooren, L. J. (1999). Transsexualism: A review of etiology, diagnosis and treatment. *Journal of Psychosomatic Research*, *46*, 315–333.

Cohen-Kettenis, P. T., van Goozen, S. H., Doorn, C. D., & Gooren, L. J. (1998). Cognitive ability and cerebral lateralisation in transsexuals. *Psychoneuroendocrinology*, *23*, 631–641.

Colapinto, J. (2001). As nature made him. The boy who was raised as a girl. New York, NY: Harper Collins Publishers Inc.

Coolidge, F. L., Thede, L. L., & Young, S. E. (2002). The heritability of gender identity disorder in a child and adolescent twin sample. *Behavior Genetics*, *32*, 251–257.

De Zegher, F., Devlieger, H., & Veldhuis, J. D. (1992). Pulsatile and sexually dimorphic secretion of luteinizing hormone in the human infant on the day of birth. *Pediatric Research*, *32*, 605–607.

DES Sons' International Research Network: http://des-sons.grouply.com/login/

Dessens, A. B., Cohen-Kettenis, P. T., Mellenbergh, G. J., vd Poll, N., Koppe, J. G., & Boer, K. (1999). Prenatal exposure to anticonvulsants and psychosexual development. *Archives of Sexual Behavior*, *28*, 31–44.

Dessens, A. B., Slijper, F. M., & Drop, S. L. (2005). Gender dysphoria and gender change in chromosomal females with congenital adrenal hyperplasia. *Archives of Sexual Behavior*, *34*, 389–397.

Diamond, M., & Sigmundson, K. (1997). Sex reassignment at birth. Long-Term review and clinical implications. *Archives of Pediatrics & Adolescent Medicine*, *151*, 298–304.

Dieckmann, G., & Hassler, R. (1977). Treatment of sexual violence by stereotactic hypothalamotomy. In W. H. Sweet, S. Obrador, & J. G. Martin-Rodriguez (Eds.), Neurosurgical

treatment in psychiatry, pain, and epilepsy (pp. 451–462). Baltimore, MD: University Park Press.

Ehrhardt, A. A., Meyer-Bahlburg, H. F., Rosen, L. R., et al. (1985). Sexual orientation after prenatal exposure to exogenous estrogen. *Archives of Sexual Behavior*, *14*, 57–77.

Ellis, L., Ames, M. A., Peckham, W., et al. (1988). Sexual orientation of human offspring May be altered by severe maternal stress during pregnancy. *Journal of Sex Research*, *25*, 152–157.

Ellis, L., & Cole-Harding, S. (2001). The effects of prenatal stress, and of prenatal alcohol and nicotine exposure, on human sexual orientation. *Physiology & Behavior*, *74*, 213–226.

Ellis, L., & Hellberg, J. (2005). Fetal exposure to prescription drugs and adult sexual orientation. *Personality and Individual Differences*, *38*, 225–236.

Fernández-Guasti, A., Kruijver, F. P., Fodor, M., & Swaab, D. F. (2000). Sex differences in the distribution of androgen receptors in the human hypothalamus. *Journal of Comparative Neurology*, *425*, 422–435.

Finegan, J. A., Bartleman, B., & Wong, P. Y. (1989). A window for the study of prenatal sex hormone influences on postnatal development. *Journal of Genetic Psychology*, *150*, 101–112.

Garcia-Falgueras, A., Pinos, H., Collado, P., et al. (2005). The role of the androgen receptor in CNS masculinization. *Brain Research*, *1035*, 13–23.

Garcia-Falgueras, A., & Swaab, D. F. (2008). A sex difference in the hypothamaic uncinate nucleus: Relationship to gender identity. *Brain*, *131*, 3132–3146.

Gender Identity Research and Education Society, information page: http://www.gires.org.uk/genderdev.php

Gizewski, E. R., Krause, E., Schlamann, M., 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. *Journal of Sexual Medicine*, *6*, 440–448.

Goldstein, J. M., Seidman, L. J., Horton, N. J., et al. (2001). Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cerebral Cortex*, *11*, 490–497.

Gómez-Gil, E., Esteva, I., Almaraz, M. C., Pasaro, E., Segovia, S., & Guillamon, A. (2010a). Familiality of gender identity disorder in non-twin siblings. *Archives of Sexual Behavior*, *39*, 546–552.

Gómez-Gil, E., Esteva, I., Carrasco, R., Almaraz, M. C., Pasaro, E., Salamero, M., et al. (2010b). Birth order and ratio of brothers to sisters in spanish transsexuals. *Archives of Sexual Behavior*, *16*, 1–6.

Gorski, R. A. (1984). Critical role for the medial preoptic area in the sexual differentiation of the brain. *Progress in Brain Research*, *61*, 129–146.

Green, R. (1978). Sexual identity of 37 children raised by homosexual or transsexual parents. *The American Journal of Psychiatry*, *135*, 692–697.

Habermeyer, E., Kamps, I., & Kawohl, W. (2003). A case of bipolar psychosis and transsexualism. *Psychopathology*, *36*, 168–170.

Hamer, D. H., Hu, S., Magnuson, V. L., Hu, N., & Pattatucci, A. M. (1993). A linkage between DNA markers on the X chromosome and male sexual orientation. *Science*, *261*, 321–327.

Hare, L., Bernard, P., Sánchez, F. J., et al. (2009). Androgen receptor repeat length polymorphism associated with male-to-female transsexualism. *Biological Psychiatry*, *65*, 93–96.

Hengstschläger, M., van Trotsenburg, M., Repa, C., Marton, E., Huber, J. C., & Bernaschek, G. (2003). Sex chromosome aberrations and transsexualism. *Fertility and Sterility*, *79*, 639–640.

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

Hines, M. (2003). Sex steroids and human behavior: Prenatal androgen exposure and sex-typical play behavior in children. *Annals of the New York Academy of Sciences*, *1007*, 272–282.

Hu, S. H., Wei, N., Wang, Q. D., et al. (2008). Patterns of brain activation during visually evoked sexual arousal differ between homosexual and heterosexual men. *American Journal of Neuroradiology*, *29*, 1890–1896.

Hughes, I. A., Houk, C., Ahmed, S. F., et al. (2006). Consensus statement on management of intersex disorders. *Archives of Disease in Childhood*, *91*, 554–563.

Iemmola, F., & Camperio Ciani, A. (2009). New evidence of genetic factors influencing sexual orientation in men: Female fecundity increase in the maternal line. *Archives of Sexual Behavior*, *38*, 393–399.

Imperato-McGinley, J., Peterson, R. E., Gautier, T., & Sturla, E. (1979). Male pseudohermaphroditism secondary to 5 alpha-reductase deficiency—a model for the role of androgens in both the development of the male phenotype and the evolution of a male gender identity. *Journal of Steroid Biochemistry*, *11*, 637–645.

Ishunina, T. A., Fischer, D. F., & Swaab, D. F. (2007). Estrogen receptor alpha and its splice variants in the hippocampus in aging and alzheimer's disease. *Neurobiology of Aging*, *28*, 1670–1681.

Ishunina, T. A., & Swaab, D. F. (2008). Estrogen receptor-alpha splice variants in the human brain. *Gynecological Endocrinology*, *24*, 93–98.

Jürgensen, M., Hiort, O., Holterhus, P. M., & Thyen, U. (2007). Gender role behavior in children with XY karyotype and disorders of sex development. *Hormones and Behavior*, *51*, 443–453.

Karama, S., Lecours, A. R., Leroux, J. M., Bourgouin, P., Beaudoin, G., Joubert, S., et al. (2002). Areas of brain activation in males and females during viewing of erotic film excerpts. *Human Brain Mapping*, *16*, 1–13.

Keenan, J. P., Wheeler, M. A., Gallup, G. G.Jr, & Pascual-Leone, A. (2000). Self-recognition and the right prefrontal cortex. *Trends in Cognitive Sciences*, *4*, 338–344.

Kindon, H. A., Baum, M. J., & Paredes, R. J. (1996). Medial preoptic/anterior hypothalamic lesions induce a female-typical profile of sexual partner preference in male ferrets. *Hormones and Behavior*, *30*, 514–527.

Kinnunen, L. H., Moltz, H., Metz, J., & Cooper, M. (2004). Differential brain activation in exclusively homosexual and heterosexual men produced by the selective serotonin reuptake inhibitor, fluoxetine. *Brain Research*, *1024*, 251–254.

Koutcherov, Y., Paxinos, G., & Mai, J. K. (2007). Organization of the human medial preoptic nucleus. *Journal of Comparative Neurology*, *503*, 392–406.

Kranz, F., & Ishai, A. (2006). Face perception is modulated by sexual preference. *Current Biology*, *16*, 63–68.

Kruijver, F. P., Balesar, R., Espila, A. M., Unmehopa, U. A., & Swaab, D. F. (2002). Estrogen receptor-alpha distribution in the human hypothalamus in relation to sex and endocrine status. *Journal of Comparative Neurology*, *454*, 115–139.

Kruijver, F. P., Balesar, R., Espila, A. M., Unmehopa, U. A., & Swaab, D. F. (2003). Estrogen-receptor-beta distribution in the human hypothalamus: Similarities and differences with ER alpha distribution. *Journal of Comparative Neurology*, *466*, 251–277.

Kruijver, F. P., Fernández-Guasti, A., Fodor, M., Kraan, E. M., & Swaab, D. F. (2001). Sex differences in androgen receptors of the human mamillary bodies are related to endocrine status rather than to sexual orientation or transsexuality. *Journal of Clinical Endocrinology and Metabolism*, *86*, 818–827.

Kruijver, F. P., & Swaab, D. F. (2002). Sex hormone receptors are present in the human suprachiasmatic nucleus. *Neuroendocrinology*, *75*, 296–305.

Kruijver, F. P., Zhou, J. N., Pool, C. W., Hofman, M. A., Gooren, L. J., & Swaab, D. F. (2000). Male-to-female transsexuals have female neuron numbers in a limbic nucleus. *Journal of Clinical Endocrinology and Metabolism*, *85*, 2034–2041.

Leedy, M. G. (1984). Effects of small medial preoptic lesions on estrous cycles and receptivity in female rats. *Psychoneuroendocrinology*, *9*, 189–196.

LeVay, S. (1991). A difference in hypothalamic structure between heterosexual and homosexual men. *Science*, *253*, 1034–1037.

LeVay, S. (1996). Queer science. The use and abuse of research into homosexuality. Cambridge, MA: MIT Press.

LeVay, S., & Hamer, D. H. (1994). Evidence for a biological influence in male homosexuality. *Scientific American*, *270*, 44–49.

Luders, E., Sánchez, F. J., Gaser, C., Toga, A. W., Narr, K. L., Hamilton, L. S., et al. (2009). Regional gray matter variation in male-to-female transsexualism. *NeuroImage*, *46*, 904–907.

MacLean, P. D., & Ploog, D. W. (1962). Cerebral representation of penile erection. *Journal of Neurophysiology*, *25*, 29–55.

Meyer-Bahlburg, H. F. (2005). Gender identity outcome in female-raised 46,XY persons with penile agenesis, cloacal exstrophy of the bladder, or penile ablation. *Archives of Sexual Behavior*, *34*, 423–438.

Meyer-Bahlburg, H. F., Ehrhardt, A. A., & Rosen, L. R. (1995). Prenatal estrogens and the development of homosexual orientation. *Developmental Psychology*, *31*, 12–21.

Meyer-Bahlburg, H. F., Gruen, R. S., New, M. I., et al. (1996). Gender change from female to male in classical congenital adrenal hyperplasia. *Hormones and Behavior*, *30*, 319–332.

Miller, B. L., Cummings, J. L., McIntyre, H., Ebers, G., & Grode, M. (1986). Hypersexuality or altered sexual preference following brain injury. *Journal of Neurology, Neurosurgery, and Psychiatry*, *49*, 867–873.

Money, J. (1975). Ablatio penis: Normal male infant sex-reassigned as a girl. *Archives of Sexual Behavior*, *4*, 65–71.

Money, J., & Erhardt, A. A. (1972). Man and woman, boy and girl: The differentiation and dimorphism of gender identity from conception to maturity. Baltimore, MD: Johns Hopkins University Press.

Morris, J. A., Jordan, C. L., Dugger, B. N., & Breedlove, S. M. (2005). Partial demasculinization of several brain regions in adult male (XY) rats with a dysfunctional androgen receptor gene. *Journal of Comparative Neurology*, *487*, 217–226.

Mouaffak, F., Gallarda, T., Baup, N., Olié, J. P., & Krebs, M. O. (2007). Gender identity disorders and bipolar disorder associated with the ring Y chromosome. *The American Journal of Psychiatry*, *164*, 1122–1123.

Mueller, A., Gooren, L. J., Naton-Schötz, S., Cupisti, S., Beckmann, M. W., & Dittrich, R. (2008). Prevalence of polycystic ovary syndrome and hyperandrogenemia in female-to-male transsexuals. *Journal of Clinical Endocrinology and Metabolism*, *93*, 1408–1411.

Mustanski, B. S., Dupree, M. G., Nievergelt, C. M., Bocklandt, S., Schork, N. J., & Hamer, D. H. (2005). A genomewide scan of male sexual orientation. *Human Genetics*, *116*, 272–278.

Northoff, G., Heinzel, A., de Greck, M., Bermpohl, F., Dobrowolny, H., & Panksepp, J. (2006). Self-referential processing in our brain–a meta-analysis of imaging studies on the self. *NeuroImage*, *31*, 440–457.

Padmanabhan, V., Manikkam, M., Recabarren, S., & Foster, D. (2005). Prenatal testosterone excess programs reproductive and metabolic dysfunction in the female. *Molecular and Cellular Endocrinology*, *246*, 165–174.

Paredes, R. G., & Baum, M. J. (1995). Altered sexual partner preference in male ferrets given excitotoxic lesions of the preoptic area/anterior hypothalamus. *Journal of Neuroscience*, *15*, 6619–6630.

Paul, T., Schiffer, B., Zwarg, T., et al. (2008). Brain response to visual sexual stimuli in heterosexual and homosexual males. *Human Brain Mapping*, *29*, 726–735.

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behaviour in the female guinea pig. *Endocrinology*, *65*, 369–382.

Ponseti, J., Bosinski, H. A., Wolff, S., Peller, M., Jansen, O., Mehdorn, H. M., et al. (2006). A functional endophenotype for sexual orientation in humans. *NeuroImage*, *33*, 825–833.

Ponseti, J., Granert, O., Jansen, O., Wolff, S., Mehdorn, H., Bosinski, H., et al. (2009). Assessment of sexual orientation using the hemodynamic brain response to visual sexual stimuli. *Journal of Sexual Medicine*, *6*, 1628–1634.

Praveen, E. P., Desai, A. K., Khurana, M. L., et al. (2008). Gender identity of children and young adults with 5alpha-reductase deficiency. *Journal of Pediatric Endocrinology & Metabolism*, *21*, 173–179.

Quigley, C. A. (2002). Editorial: The postnatal gonadotropin and sex steroid surge—insights from the androgen insensitivity syndrome. *Journal of Clinical Endocrinology and Metabolism*, *87*, 24–28.

Rahman, Q., Abrahams, S., & Wilson, G. D. (2003). Sexual-orientation-related differences in verbal fluency. *Neuropsychology*, *17*, 240–246.

Rahman, Q., & Koerting, J. (2008). Sexual orientation-related differences in allocentric spatial memory tasks. *Hippocampus*, *18*, 55–63.

Rametti, G., Carrillo, B., Gómez-Gil, E., Junque, C., Segovia, S., Gomez, A., et al. (2010). White matter microstructure in female to male transsexuals before cross-sex hormonal treatment. A diffusion tensor imaging study. *Journal of Psychiatric Research*, *8*, 1–6.

Reiner, W. G. (1999). Assignment of sex in neonates with ambiguous genitalia. *Current Opinion in Pediatrics*, *11*, 363–365.

Reiner, W. G. (2005). Gender identity and sex-of-rearing in children with disorders of sexual differentiation. *Journal of Pediatric Endocrinology & Metabolism*, *18*, 549–553.

Reiner, W. G., & Gearhart, J. P. (2004). Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth. *The New England Journal of Medicine*, *350*, 333–341.

Savic, I. (2005). Brain imaging studies of the functional organization of human olfaction. *Chemical Senses*, *30*, 222–223.

Savic, I., Berglund, H., Gulyas, B., & Roland, P. (2001). Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans. *Neuron*, *31*, 661–668.

Savic, I., Berglund, H., & Lindström, P. (2005). Brain response to putative pheromones in homosexual men. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 7356–7361.

Savic, I., & Engel, J. Jr. (1998). Sex differences in patients with mesial temporal lobe epilepsy. *Journal of Neurology, Neurosurgery, and Psychiatry*, *65*, 910–912.

Savic, I., & Lindström, P. (2008). PET and MRI show differences in cerebral asymmetry and functional connectivity between homo- and heterosexual subjects. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 9403–9408.

Schober, J. M., & Pfaff, D. (2007). The neurophysiology of sexual arousal. *Best Practice & Research. Clinical Endocrinology & Metabolism*, *21*, 445–461.

Schwarz, J. M., Nugent, B. M., & McCarthy, M. M. (2010). Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span. *Endocrinology*, *151*, 1–11.

Spitzer, R. L. (2003). Can some gay men and lesbians change their sexual orientation? 200 participants reporting a change from homosexual to heterosexual orientation. *Archives of Sexual Behavior*, *32*, 403–492.

Swaab, D. F. (2003). The human hypothalamus. Basic and clinical aspects. Part I: Nuclei of the hypothalamus. In M. J. Aminoff, F. Boller, & D. F. Swaab (Eds.), Handbook of clinical neurology (pp. 127–140). Amsterdam, The Netherlands: Elsevier.

Swaab, D. F. (2004). The human hypothalamus. Basic and clinical aspects. Part II: Neuropathology of the hypothalamus and adjacent brain structures. In M. J. Aminoff, F. Boller, D. F. Swaab (Eds.), Handbook of clinical neurology (pp. 193–231). Amsterdam, The Netherlands: Elsevier.

Swaab, D. F. (2008). Sexual orientation and its basis in brain structure and function. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 10273–10274.

Swaab, D. F., Chung, W. C., Kruijver, F. P., Hofman, M. A., & Hestiantoro, A. (2003). Sex differences in the hypothalamus in the different stages of human life. *Neurobiology of Aging*, *1*, S1–S19.

Swaab, D. F., Chung, W. C., Kruijver, F. P., Hofman, M. A., & Ishunina, T. A. (2001). Structural and functional sex differences in the human hypothalamus. *Hormones and Behavior*, *40*, 93–98.

Swaab, D. F., & Fliers, E. (1985). A sexually dimorphic nucleus in the human brain. *Science*, *228*, 1112–1115.

Swaab, D. F., Gooren, L. J., & Hofman, M. A. (1992). The human hypothalamus in relation to gender and sexual orientation. *Progress in Brain Research*, *93*, 205–219.

Swaab, D. F., & Hofman, M. A. (1984). Sexual differentiation of the human brain. A historical perspective. *Progress in Brain Research*, *61*, 361–374.

Swaab, D. F., & Hofman, M. A. (1988). Sexual differentiation of the human hypothalamus: Ontogeny of the sexually dimorphic nucleus of the preoptic area. *Brain Research. Developmental Brain Research*, *44*, 314–318.

Swaab, D. F., & Hofman, M. A. (1990). An enlarged suprachiasmatic nucleus in homosexual men. *Brain Research*, *537*, 141–148.

Swaab, D. F., Slob, A. K., Houtsmuller, E. J., Brand, T., & Zhou, J. N. (1995). Increased number of vasopressin neurons in the suprachiasmatic nucleus (SCN) of "bisexual" adult male rats following perinatal treatment with the aromatase blocker ATD. *Brain Research. Developmental Brain Research*, *85*, 273–279.

Swan, S. H., Liu, F., Hines, M., Kruse, R. L., Wang, C., Redmon, J. B., et al. (2010). Prenatal phthalate exposure and reduced masculine play in boys. *International Journal of Andrology*, *33*, 259–269.

Titus-Ernstoff, L., Perez, K., Hatch, E. E., et al. (2003). Psychosexual characteristics of men and women exposed prenatally to diethylstilbestrol. *Epidemiology*, *14*, 155–160.

van Beijsterveldt, C. E., Hudziak, J. J., & Boomsma, D. I. (2006). Genetic and environmental influences on cross-gender behavior and relation to behavior problems: A study of dutch twins at ages 7 and 10 years. *Archives of Sexual Behavior*, *35*, 647–658.

van de Beek, C., van Goozen, S. H., Buitelaar, J. K., & Cohen-Kettenis, P. T. (2009). Prenatal sex hormones (maternal and amniotic fluid) and gender-related play behavior in 13-month-old infants. *Archives of Sexual Behavior*, *38*, 6–15.

Vreugdenhil, H. J., Slijper, F. M., Mulder, P. G., & Weisglas-Kuperus, N. (2002). Effects of perinatal exposure to PCBs and dioxins on play behavior in dutch children at school age. *Environmental Health Perspectives*, *110*, A593–A598.

Wallien, M. S., & Cohen-Kettenis, P. T. (2008). Psychosexual outcome of gender-dysphoric children. *Journal of the American Academy of Child and Adolescent Psychiatry*, *47*, 1413–1423.

Wilson, J. D., Griffin, J. E., & Russell, D. W. (1993). Steroid 5 alpha-reductase 2 deficiency. *Endocrine Reviews*, *14*, 577–593.

Wisniewski, A. B., Migeon, C. J., Meyer-Bahlburg, H. F., et al. (2000). Complete androgen insensitivity syndrome: Long term medical, surgical, and psychosexual outcome. *Journal of Clinical Endocrinology and Metabolism*, *85*, 2664–2669.

Witelson, S. F., Kigar, D. L., Scamvougeras, A., Kideckel, D. M., Buck, B., Stanchev, P. L., et al. (2008). Corpus callosum anatomy in right-handed homosexual and heterosexual men. *Archives of Sexual Behavior*, *37*, 857–863.

Zhou, J. N., Hofman, M. A., Gooren, L. J., & Swaab, D. F. (1995). A sex difference in the human brain and its relation to transsexuality. *Nature*, *378*, 68–70.

Zucker, K. J., Bradley, S. J., Oliver, G., Blake, J., Fleming, S., & Hood, J. (1996). Psychosexual development of women with congenital adrenal hyperplasia. *Hormones and Behavior*, *30*, 300–318.

Zufall, F. (2005). Connexins and olfactory synchronicity: Toward the olfactory code. *Neuron*, *46*, 693–694.

Zuloaga, D. G., Puts, D. A., Jordan, C. L., & Breedlove, S. M. (2008). The role of androgen receptors in the masculinization of brain and behavior: What we've learned from the testicular feminization mutation. *Hormones and Behavior*, *53*, 613–626.