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Sexual differentiation of the human brain: relevance for gender identity, transsexualism and sexual orientation

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ABSTRACT

Male sexual differentiation of the brain and behavior are thought, on the basis of experiments in rodents, to be caused by androgens, following conversion to estrogens. However, observations in human subjects with genetic and other disorders show that direct effects of testosterone on the developing fetal brain are of major importance for the development of male gender identity and male heterosexual orientation. Solid evidence for the importance of postnatal social factors is lacking. In the human brain, structural differences have been described that seem to be related to gender identity and sexual orientation.

MECHANISM OF SEXUAL DIFFERENTIATION OF THE BRAIN: THE RODENT IS NOT A GOOD MODEL

Sexual differentiation of the brain is thought to be 'imprinted' or 'organized' by hormonal signals from the developing male gonads. On the basis of animal experiments, it is presumed that this process is induced by androgens during development, following conversion to estrogens by P-450 aromatase. All these components are also present in the human brain. Aromatase is present throughout the brain, including the hypothalamus, of both men and women[1,2], as are estrogen receptors (ER) α and β[3,4]. Male sexual differentiation of the human brain is thought to be determined in the two first periods during which sexually dimorphic peaks in gonadal hormone levels are found – during gestation and the perinatal period, while from puberty onwards, sex hormones alter the function of previously organized neuronal systems ('activating effects')[5–7]. Studies in primates indicate that the period of the neonatal testosterone peak is critical in the process of sexual and behavioral development[8]. However, observations in male subjects with cloacal exstrophy, who were sex-reassigned to females at birth and later declared themselves male, indicate that it is the prenatal testosterone surge that is most important for the development of gender identity[9]. The importance of the fetal male testosterone surge for sexual differentiation of the brain following aromatization to estrogens agrees with the observations that girls whose mothers were exposed to diethylstilbestrol (DES) during pregnancy run a higher risk of developing bi- or homosexuality[10,11].

Although estrogens, derived from testosterone by aromatization, are considered to be the major mediator for androgenization of the brain during

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development in rodents, testosterone itself may be of major importance for sexual differentiation of the human brain for several reasons. First, both sexes are exposed to high levels of estrogens during fetal life, while it is only males who are subjected to high androgen levels[12]. In human neonates the testosterone level is tenfold higher in males than in females at 34–41 weeks of gestation[6]. Although the peak in serum testosterone in male infants 1–3 months postnatally approaches the levels seen in adult men, most testosterone is bound to globulin. Yet the amount of free testosterone in male infants is about one order of magnitude larger than in female infants at this time[13]. Moreover, the androgen receptor (AR), located on the X chromosome at Xq11–12, may be mutated in such a way that the subject has complete androgen-insensitivity syndrome. In spite of normal testis differentiation and androgen biosynthesis, the phenotype has a normal female external and behavioral appearance[14,15]. Phenotypic women with complete androgen-insensitivity syndrome perceive themselves as highly feminine. They do not have gender problems and largely report their sexual attraction, fantasies and experiences as being female and heterosexual[16,17]. This means that, for the development of human male gender identity and male heterosexuality, direct androgen action on the brain seems to be of crucial importance, and the aromatization theory as derived from experiments in rodents may be of secondary importance for sexual differentiation of the human brain. Experiments in rodents and non-human primates now also indicate the importance of androgens for the masculinization of the brain, not only by regulating transcription of aromatase mRNA[18,19] but also by a direct effect on the AR[19,20].

MUTATIONS AND GENDER

The viewpoint that aromatization may be less important than previously thought agrees with reports of a lack of gender problems in a brother and sister with aromatase deficiency due to an inactivating mutation of the *P450* gene and in a 27-year-old man with a mutation of the *CYP* gene (of which aromatase cytochrome is a product). Both cases were accompanied by psychosexual orientation appropriate for their genetic and phenotypic sex[21–26]. Moreover, a 28-year-old man with estrogen resistance due to a mutation of the *ER* gene was described as tall, with continued linear growth in adulthood, incomplete epiphysal closure and increased estrogen and gonadotropin levels. A change in a single base pair in the second exon of the *ER* gene was found. However, the patient reported no history of gender-identity disorder, had strong heterosexual interest and normal male genitalia.

Moreover, the heterosexual XY females resulting from the complete androgen-insensitivity syndrome[17] and the male gender heterosexual behavior of patients with 5α-reductase-2 or 17β-hydroxysteroid dehydrogenase-3 deficiency[27–30] indicate that a direct action of testosterone may be more important than that of dihydrotestosterone (DHT) for male heterosexual psychosexual development. The latter affected 46,XY individuals have normal to elevated to high plasma testosterone levels with decreased DHT levels. They have ambiguous external genitalia at birth, so that they are often raised as girls. Virilization occurs at puberty, frequently with a gender role change. These subjects demonstrate that exposure of the brain to testosterone during development and at puberty appears to have a greater impact in determining male gender identity than do sex of rearing and sociocultural influences[31].

SEX DIFFERENCES IN ANDROGEN RECEPTOR

In most hypothalamic areas that stain positively for the AR, nuclear staining in particular is less intense in young adult women than in men. The strongest sex difference is found in the lateral and medial mamillary nucleus[32]. The mamillary body complex is known to receive input from the hippocampus by the fornix and to be involved in cognition. Moreover, this complex is also involved in several aspects of sexual behavior, such as penile erection (see below). In addition, a sex difference in AR staining is present in the horizontal diagonal band of Broca, the sexually dimorphic nucleus of the preoptic area, the medial preoptic area, the dorsal and ventral zone of the periventricular nucleus, the paraventricular nucleus, the supraoptic nucleus, the ventromedial hypothalamic nucleus and the infundibular nucleus. No sex differences were observed in AR staining in the bed nucleus of the stria terminalis, the nucleus basalis of Meynert and the island of Calleja[32]. Nuclear AR activity in the mamillary complex of heterosexual men did not differ from that of homosexual men, but it was significantly stronger in men than in women. A female-like pattern was

found in men with low testosterone levels: e.g. in two castrated male-to-female transsexuals; in two castrated men, one 26 years old and the other 53 years old; and in intact elderly men. These data indicate that the amount of nuclear AR staining in the mamillary complex is dependent on the circulating levels of androgens, rather than on gender identity or sexual orientation. This idea is supported by the finding that a male-like pattern of AR staining was found in a 36-year-old, bisexual, non-castrated male-to-female transsexual and in a heterosexual, virilized, 46-year-old woman[33].

The relative contributions of the different sex hormones and other non-hormonal factors on sexual differentiation of the human brain should clearly be a focus for future research, including a more detailed endocrine and psychosexual investigation of patients with mutations in aromatase or sex hormone receptors.

GENETIC CONTROL OF SEXUAL DIFFERENTIATION OF THE BRAIN

It is of great interest that, in addition, there is recent animal experimental evidence for primary genetic control of sexual differentiation that does not involve sex hormones. Results obtained from cultures of embryonic rat brain indicate that dopaminergic neurons may develop morphological and functional sex differences in the absence of sex steroids[34]. Candidates for such hormone-independent effects are those genes located on the non-recombining part of the Y chromosome and believed to be involved in primary sex determination of the organism. Two candidate genes are the two testis-determining factors, *ZFY* and the master switch for differentiation of a testis *SRY*; they are putative transcription factors. We have shown that *SRY* and *ZFY* are transcribed in the hypothalamus and frontal and temporal cortex of adult men, and not in women. It may well be possible that they function as sex-specific cell-intrinsic signals that are needed for full differentiation of a male human brain, and that continuous expression throughout life may be required to maintain sex-specific structural or functional properties of differentiated male neurons. Sexual differentiation of the human brain may thus be a multi-factorial process, although a role of *SRY* and *ZFY* in this process still needs to be proved[35]. Recent microarray studies in mice have shown that there are over 50 genes expressed in a sexually dimorphic way in the brain before gonadal differentiation takes place[36]. The relative contributions of the different sex hormones and other non-hormonal factors on sexual differentiation of the human brain should clearly be a focus for future research.

GENDER-IDENTITY PROBLEMS AND TRANSSEXUALITY

The transsexual is characterized by an unshakable conviction of belonging to the opposite sex, presenting a most extreme gender-identity disorder. Gender identity (gender identity refers to an identity experience expressed in terms of masculine or feminine 'belongingness', independent of the anatomical reality of the sex) is therefore totally in disharmony with corporal reality, forcing the individual to request sex-reassignment surgery. Gender-related traits may also resemble those of the opposite sex in transsexuals[37].

Genetic factors and gender

There is little information about the factors that may influence gender and cause gender-identity disorders and transsexuality in humans[38] (Table 1). For gender-identity disorder in early development, a strong (62%) hereditary component was found on the basis of twin studies[47]. The disparate maternal aunt to uncle ratio in male transsexuals has been hypothesized to be due to genomic imprinting[48]. There are only a few reports that have found chromosomal abnormalities in transsexuals. Six cases of male-to-female transsexuals with 47,XYY chromosome and one female-to-male transsexual with 47,XXX have been reported[40]. A phenotypically female baby with 46,XY sex reversal, persisting Müllerian structures and a primary adrenal failure was reported to have a mutation of steroidogenic factor-1. Subsequently a heterozygous frameshift mutation has been described with 46,XY sex reversal and cliteromegaly, but no uterus, raising the possibility that other mutations of this transcription factor might have milder or tissue-specific effects in humans. So far these concern complete sex reversal and not transsexuality[42,43]. Moreover, transsexualism has been reported in a Kleinfelter (XXY) male[46]. In addition, pairs of monozygotic female twins have requested sex reassignment, and twin studies and familial cases of gender-identity problems are reported, suggesting a genetic basis for

Table 1 Factors that influence gender identity (transsexualism). Modified from reference 39

Chromosomal disorders
rare: 47,XYY (male-to-female), 47,XXX (female-to-male); microdeletion on Y chromosome in one male-to-female transsexual[40,41]
steroidogenic factor-1 mutations (give sex reversal, no transsexuality)[42–45]
Klinefelter XXY male-to-female[46]
twin studies[46,47]
genomic imprinting[48]
Phenobarbital/diphantoin[49]
Hormones
intersex[50,51], micropenis[52]
cloacal exstrophy[9,53,54]
5α-reductase deficiency, 17β-hydroxysteroid dehydrogenase-3 deficiency[27,28,30]
CAH girls with gender problems[55–57]
more polycystic ovaries, oligomenorrhea and amenorrhea are found in transsexuals[58]
complete androgen-insensitivity syndrome results in XY heterosexual females[17]
Social factors?[59]
not effective: John/Joan/John case[38,60]

CAH, congenital adrenal hyperplasia

this disorder[46,47,61]. Genetic aberrations, however, are generally undetectable in transsexuals when G-banded karyotypes are analyzed, and evidence has been presented that molecular cytogenetic alterations affecting the *AR* gene region or the *SRY* region do not play a role in transsexualism. One carrier of a microdeletion on the Y chromosome has been detected in a series of 30 male-to-female transsexuals[41]. The claim of Dörner and colleagues[62], that in transsexuals a partial 21-hydroxylase deficiency may be present, has yet to be confirmed.

Hormones and gender

Although only a minority of transsexuals have an underlying endocrine abnormality[63], there are some indications of a possible disorder of the hypothalamus–pituitary–gonadal axis in some transsexuals that may have a basis in development, such as the high frequency of polycystic ovaries, oligomenorrhea and amenorrhea in female-to-male transsexualism[46,58]. These observations may be explained by a difference in the interactions between hormones and the developing brain. Dessens and associates[49] have reported that three children born of a group of 243 women exposed to the anticonvulsants phenobarbital and diphantoin were found to be transsexuals, while there were a few other subjects with gender dysphoria/cross-gender behavior. Gender problems thus occur remarkably often, in view of the rarity of this disorder. This exciting observation on the effect of compounds that are known to alter steroid hormone levels in animal experiments has to be examined further. In this respect, it is of interest to note that phenobarbital has been widely used as prophylactic treatment in neonatal jaundice and greatly elevates the postnatal rise in testosterone[64]. There has not, however, been a follow-up on gender-identity disorders so far. Also, endocrine disrupters present in food and water may be considered with respect to gender-identity problems, such as veterinary growth promoters and resveratrol, a phytoestrogen that is present in grapes and wine and that is an agonist for the ER[65,66]. Long-term effects of endocrine disrupters on human brain and behavior differentiation have, however, not been studied so far. In 1996, Meyer-Bahlburg and co-workers[55] reported a gender change from female to male in four 46,XX individuals with classic congenital adrenal hyperplasia. Congenital adrenal hyperplasia, characterized by high androgen levels during prenatal development in 90% due to a defect in 21-hydroxylase, indeed constitutes a risk factor for the development of gender-identity problems[56]. However, others found only a small increase in risk of atypical gender identity in girls with congenital adrenal hyperplasia[57] or, in a small group, an absence of gender-identity dysphoria[67]. Thus although it should be emphasized that the majority of women with this disorder may not experience a marked gender-identity conflict, the odds that a genetic female with this disease would live, as an adult, in the

male social role was found to be 608:1, compared with genetic females in the general population[68]. These observations support the view that intrauterine or perinatal exposure to abnormal levels of sex hormones might permanently affect gender identity. The finding that both male and female transsexuals were more often not right-handed than controls is also consistent with the theory of altered prenatal sex hormone origin for transsexualism[69].

Reiner[50] described a 46,XY child with mixed gonadal dysgenesis, one immature testis, hypoplastic uterus and clitoral hypertrophy, who was raised without stigmatization as a girl, but who declared himself male at the age of 14. Following corrective surgery and testosterone substitution, he lived as a boy despite the social factors that were clearly in favor of maintaining the assigned sex. Apparently the deficient testis had been able to organize the brain during development, even though the hormone levels were prenatally so inadequate that ambiguity of the genitalia was induced. A child with true hermaphroditism and 45,X (13%) 47,XYY (87%) sex chromosome mosaic pattern in blood, uterus, fallopian tubes, phallus, testicular tissue and epididymis was assigned the male sex at birth. At 5 weeks the decision was made to reassign him to female. At 9 months an operation was performed to make the genitalia female, at 13 months the testicle was removed and at 5 years another operation was done to make the genitalia female. She was raised as a girl but had masculine interests, and around 8 years of age she declared that 'God had made a mistake' and that she 'should have been a boy'. Apparently the male sex hormones to which she had been exposed *in utero* had imprinted the male gender, although the authors also presumed postnatal psychosocial factors to have played a role[61].

Social factors and gender

The concept of sexual neutrality at birth, after which infants differentiate as masculine or feminine as a result of social experiences, was proposed by Money and colleagues[70,71]. Gender imprinting was presumed to start at the age of 1 year and to be already well established by 3–4 years of age[72]. Observations on children with male pseudohermaphroditism due to 5α-reductase-2 deficiency were supposed to support the influence of life experience on psychosexual make-up[73]. However, the conclusion in the available literature is that there is no solid evidence for parental influences on the etiology of transsexuality[38]. A classic report strongly influencing the opinion that the environment plays a crucial role in gender development was that described by Money of a boy whose penis was accidentally ablated at the age of 8 months, during a phemosis repair by cautery, and who was subsequently raised as a female. Orchidectomy followed within a year to facilitate feminization and further surgery to fashion a full vagina was performed later. Initially this individual was described as developing into a normally functioning female. Later, however, it appeared that the individual had rejected the sex of raising and switched at puberty to living as a male again and requested male hormone shots, a mastectomy and phalloplasty. At the age of 25 he married a woman and adopted her children. This famous John/Joan/John story, although it is just one case, illustrates that there is little – if any – support for the view that individuals are sexually neutral at birth and that normal psychosexual development is crucially dependent on the environment[60]. Sadly, this story ended with the suicide of 'John' in May 2004. In a second case of penile ablation, in which the decision was made to reassign the patient to female and raise the baby as a girl, the remainder of the penis and testes were removed at a slightly earlier stage (7 months). Although her adult sexual orientation was bisexual and even though she was mainly attracted to women, her gender identity was female. The authors explain the different outcome compared with the former case on the basis of the decision to reassign the sex at a younger age[59]. However, male patients with cloacal exstrophy have a herniation of the urinary bladder and hindgut, and the anatomy leaves them aphallic in the majority of the cases although the testicles are histologically normal. In a group of eight male patients who were gender-reassigned as females and orchidectomized in the neonatal period, gender identity has been questioned by the patients themselves in at least three instances[53], supporting the early programming of gender identity by biological factors and arguing against a dominating role of the social environment.

The observations that, in a longitudinal series of 16 hormonally normal 46,XY males assigned to female sex-of-rearing at birth due to the absence of a penis, eight have spontaneously declared themselves male and 15 fall very close to the male-typical spectrum of gender roles[52] lead to the same conclusion. In a follow-up of this study, eight of

the 14 male subjects that were sex-reassigned as females declared themselves males. This study also indicates that the prenatal androgens are the major biological factor for the development of male gender identity, even in the absence of the neonatal and pubertal androgen surges[9].

Despite sex assignment, correction of the genitalia soon after birth, psychological counseling of parents and intensive psychotherapy, 13% of the intersex children in the study of Slijper and associates[56] developed a gender-identity disorder. Yet only one girl (2%) failed to accept the assigned sex. The Imperato-McGinley syndrome, based upon DHT deficiency due to 5α-reductase-2 deficiency, also shows that exposure to testosterone during development has a greater impact on male gender identity than the sex of rearing and socio-cultural influences[31].

Female structures in male brains and vice versa

We have found a female-sized nucleus in the sexually dimorphic central subdivision of the bed nucleus of the stria terminalis (BSTc) in male-to-female transsexuals. These data were confirmed by neuronal counts of somatostatin cells, the major neuron population in the BSTc and total cell number in the BSTc (Figures 1 and 2). Changes in adult hormone levels could not explain this difference[74–76]. These observations support the hypothesis that gender identity develops as a result of an interaction between the developing brain and sex hormones. Much to our surprise, however, the sex difference in BSTc volume did not become overt until adulthood[76].

The explanation for the discrepancy between the late occurrence of a sex difference in the volume of this nucleus and the early occurrence of gender problems in transsexualism necessitates further research. It is possible that functional sex differences in the BSTc may precede the structural sex differences in the course of development.

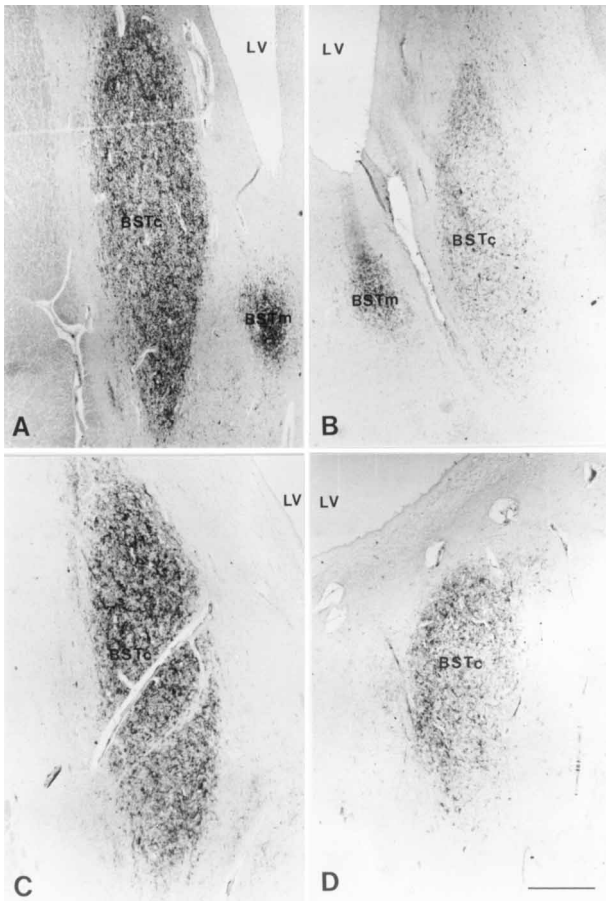

Figure 1 Representative sections of the sexually dimorphic central subdivision of the bed nucleus of the stria terminalis (BSTc) innervated by vasoactive intestinal polypeptide (VIP): (A) heterosexual man; (B) hetero-sexual woman; (C) homosexual man; (D) male-to-female transsexual. Scale bar, 0.5 mm. LV, lateral ventricle. Note there are two parts of the BST in (A) and (B): small medial subdivision (BSTm) and large oval-sized central subdivision (BSTc). Note also the sex difference (A vs. B) and the fact that the male-to-female transsexual (D) has a female BSTc in size and type of innervation (from reference 74, Figure 2, with permission)

HOMOSEXUALITY

Genetic factors and sexual orientation

Sexual orientation is influenced by quite a number of genetic as well as non-genetic factors (Table 2). Genetic factors appear from studies in families, twins and through molecular genetics[77–80,84,91,92.] Hamer and colleagues found linkage between DNA markers on the X chromosome and male sexual orientation. Genetic linkage between microsatellite markers on the X chromosome, i.e. Xq28, was detected for the gay male families, but not for the lesbian families[79,80]. In a follow-up study, Rice and co-investigators[81] studied by four markers the sharing of alleles at position Xq28 in 52 Canadian, gay male sibling pairs. Allele and haplotype sharing

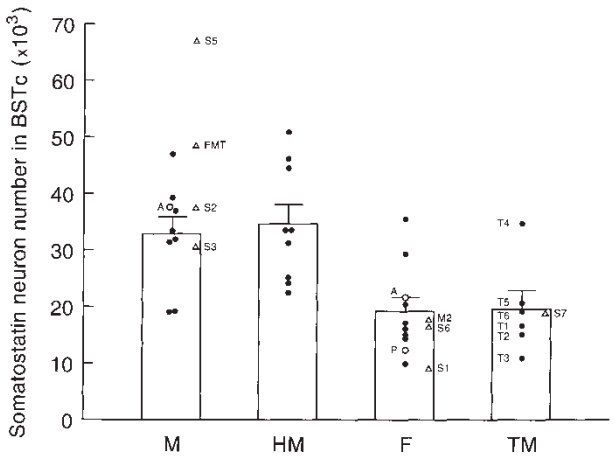

Figure 2 Neuron numbers in the sexually dimorphic central subdivision of the bed nucleus of the stria terminalis (BSTc). Distribution of the BSTc neuron numbers among different groups according to sex, sexual orientation and gender identity. M, heterosexual male reference group; HM, homosexual male group; F, female group; TM, male-to-female transsexuals. 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. The difference between the M and the TM group ($p < 0.04$) also becomes statistically significant according to the sequential Bonferonni method if S2, S3 and S5 are included in the M group or if S7 is included in the TM group ($p \leqslant 0.01$). Note that the number of neurons of the female-to-male transsexual (FMT) is fully in the male range. A, AIDS patient. BSTc neurons number in the heterosexual man and woman with AIDS remained well within the corresponding reference group, so AIDS did not seem to affect the somatostatin neuron numbers in the BSTc. P, postmenopausal woman. S1 (25 years of age), Turner syndrome (45,X0; ovarian hypoplasia). M2 (73 years of age), postmenopausal status (from reference 75, Figure 1, with permission)

for these markers was not increased more than expected, which did not support the presence of an X-linked gene underlying male homosexuality. In a reaction to this paper, Hamer[93] stated that the family pedigree data from the Canadian study supported his hypothesis, that three other available Xq28 DNA studies found linkage also, and that the heritability of sexual orientation is supported by substantial evidence independent of the X chromosome data. In a meta-analysis of the four available studies, he found a significant linkage. Rice and colleagues[93] responded extensively and remained convinced that an X-linked gene could not exist in the population with any sizeable frequency. This controversy will undoubtedly continue for a while longer. The claim of Dörner and co-workers[62], that in homosexual men a partial 3β-hydroxysteroid dehydrogenase deficiency may be present, has to be confirmed. Aromatase cytochrome P450 (*CYP19*), which is necessary for the conversion of androgen to estrogen, was studied as a candidate gene for male in homosexual brothers. However, the study revealed no indication that variation in this gene may be a major factor for the development of male homosexuality[94].

Hormones and other factors and sexual orientation

Sex hormones during development also have an influence on sexual orientation, as appears from the increased proportion of bi- and homosexual girls with congenital adrenal hyperplasia[55,82,83]. Then there is DES, a compound related to estrogens that increases the occurrence of bi- or homosexuality in girls whose mothers received DES during pregnancy[10,11,84] to prevent miscarriage (*quod non*). DES was given between 1939 and the 1960s to millions of pregnant women[95]. However, these authors could not confirm an increase in the likelihood of homosexual behavior in DES-exposed girls or boys in adulthood. The ratio of the second to fourth finger digits, a measure ascribed to the organizational actions of prenatal androgens, was significantly lower in the homosexual males and females as compared with heterosexuals. This observation suggests that homosexual males and females have been exposed to elevated levels of androgens *in utero*[96]. Whether environmental estrogens from plastics can influence sexual differentiation of the human brain and behavior is, at present, in debate but certainly not established. However, the observation that masculine behavior increases in boys with number of years of maternal sport fish consumption[97] is consistent with this possibility. In addition, phytoestrogens, such as resveratrol, present in grapes and wine, and an agonist for the ER, should be considered in this respect[65].

Homosexual orientation is most likely to occur in men with a high number of older brothers and a shorter stature. One biological mechanism that could explain this phenomenon is an immune response in women pregnant with successive male fetuses[86].

Table 2 Factors that influence sexual orientation (homosexuality, heterosexuality). Modified from reference 39

Genetic factors
twin studies[77,78]
molecular genetics[79–80], however see[81,93]
Hormones
CAH girls[68,82,83]
diethylstilbestrol[10,11]
male-to-female sex reassignment[84]
Chemicals
nicotine prenatally increases the probability of lesbianism[85]
Immune response?
homosexual orientation in men is most likely to occur in those with a high number of older brothers and shorter stature[86]
Social factors?
stress during pregnancy[85,87,88]
raising by transsexual or homosexual parents does not affect sexual orientation[89,90]

CAH, congenital adrenal hyperplasia

Prenatal nicotine exposure has masculinizing/defeminizing effects on sexual orientation of female offspring and increases the probability of lesbianism[85].

Social factors and sexual orientation

Maternal stress is thought to lead to increased occurrence of homosexuality in boys, particularly when the stress occurs during the first trimester[85,87], and girls[88]. Weyl[98] has mentioned two interesting case histories of this prenatal environmental factor. Marcel Proust's mother was subjected to the overwhelming stress of the Paris commune during the fifth month of her pregnancy in 1871; and Mary Queen of Scots, mother of the homosexual king of England James I, had the terrifying experience, toward the end of the fifth month of pregnancy, that her secretary and special friend Riccio was killed. Although postnatal social factors are generally presumed to be involved in the development of sexual orientation[68,99], solid evidence in support of such an effect has not yet been reported. The observation that children raised by lesbian couples or by transsexuals generally have a heterosexual orientation[89,90,100] does not support the possibility of the social environment in which the child is raised as an important factor for determining sexual orientation, nor is there scientific support for the idea that homosexuality has psychoanalytical or other psychological or social learning explanations, or that it would be a 'lifestyle choice'[101]. Various hypothalamic structures are structurally different in relation to sexual orientation, i.e. the suprachiasmatic nucleus, the third interstitial nucleus of the anterior hypothalamus and the commissura anterior[102], suggesting that a difference in hypothalamic neuronal networks that occurs in development may be the basis of differences in sexual orientation.

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