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The biology of human psychosexual differentiation

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Abstract

Most attempts to identify biological underpinnings of gender identity and sexual orientation in humans have investigated effects of sex steroids, so pivotal in the differentiation of the genitalia, showing strong parallels between animals and the human. The information on humans is derived from the so-called 'experiments of nature', clinical entities with a lesser-than-normal androgen exposure in XY subjects and a higher than normal androgen exposure in XX subjects. Prenatal androgenization appears to predispose to a male gender identity development, but apparently not decisively since 40–50% of 46,XY intersexed children with a history of prenatal androgen exposure do not develop a male gender identity. Obviously, male-to-female transsexuals, with a normal androgen exposure prenatally (there is no serious evidence to the contrary) develop a female gender identity, through unknown biological mechanisms apparently overriding the effects of prenatal androgens. The latest studies in 46, XX subjects exposed to prenatal androgens show that prenatal androgenization of 46,XX fetuses leads to marked masculinization of later gender-related behavior but does not lead to gender confusion/dysphoria. The example of female-to-male transsexuals, without evidence of prenatal androgen exposure, indicates that a male gender identity can develop without a significant androgen stimulus. So we are far away from any comprehensive understanding of hormonal imprinting on gender identity formation. Brain studies in homosexuals have not held up in replication studies or are in need of replication in transsexuals.

Genetic studies and the fraternal birth order hypothesis provide indications of familial clustering of homosexuality but in many homosexuals these genetic patterns cannot be identified. The biological explanations advanced for the birth order hypothesis lack any experimental support.
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Introduction

For us human beings, the division into two sexes is one of the great eternal verities. The difference between the sexes is experienced as absolute. Subjectively, man and woman are opposites, poles, mutually exclusive forms of human existence. Wild and domesticated animals, and even plants, are, like the human race, either male or female. This has reinforced the idea that manhood and womanhood are expressions of a natural order. Since times immemorial parents have assigned their newborns to that sex that the morphology of the external genitalia indicated. This time-honored practice impresses as reasonable, since babies appearing boys and girls at birth generally grow up to become normally functioning adult men and women. In other words, no major conscious (pedagogic) effort has to be made to raise baby boys to men and baby girls to women. Manhood and womanhood would seem to be intrinsic, biologically determined, preprogrammed properties of boys and girls awaiting their completion by the hormones of puberty, stressing the already present sex differences and heralding the erotosexual interaction between the two sexes. The latter is pivotal for survival of the species and would seem to be an inherent, biologically ordained property of living organisms.

The above perception of manhood and womanhood as being complementary and necessary for survival of the species has led to the belief that the full spectrum of masculinity and femininity is the product of biological determinism rather than being (co)shaped by sociopsychological factors. The equation of sexuality with procreation has reinforced the belief that man and woman are two poles, the one does not exist without the other, they presuppose one another, but they are mutually exclusive.

The human species attributes major significance to the status of being male or female and their sexual interaction, and as a

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result, they are governed by a multitude of prescripts and proscriptions of a societal, legal and religious nature.

Anthropological research and sociological inquiry have questioned the mutual exclusivity of being man or woman. Cross-cultural studies have revealed that certain sex differences are sex arbitrary and capricious: codes for dressing, hairdo, and make up differ between societies, eras and even within the same society between social classes/subcultures. Other male–female differences are sex-adjunctive: in the history of mankind, it is intelligible that the on average greater physical strength of the male and the constraints of pregnancy and lactation became the basis of sex-adjunctive division of labor. The 20th century technology of both labor and birth control and child nursing have changed these social sex roles profoundly. Another field of study was the manifestations of sexual behavior. Anthropological and social research found an impressive diversity in sexual behavior, with homosexual behavior best studied, in different eras and different societies or societal layers. These researchers were led to believe that expressions of sexual behavior are shaped by sociocultural forces, which, in their view, are irreconcilable with biological underpinnings of such behavior. The tacit assumption was that biological underpinnings were deterministic and restrictive not allowing the pluriformity of sexual behavior encountered in their research. Most of these researchers were little knowledgeable in the area of the biological sciences and did not take notice that the outcome of biological research did not contradict their findings and actually suggested that the brain has a great adaptive quality and plasticity to meet demands that place and time impose on the species.

Transsexualism: the subjective experience

Transsexualism is the development of a gender identity that is at variance with morphology of genitals and secondary sex characteristics. John Money (1991, 1981) has defined gender identity as follows: “Gender identity is one’s own categorization of one’s individuality as male, female, or ambivalent as experienced in self-awareness of one’s own mental processes and one’s own actual behavior. Gender role is the public manifestation of one’s gender identity, the things that one says and that one does that gives people a basis for inferring whether one is male, female, or fits neither of those categories”. Money views gender identity and gender role as two aspects of the same thing, two sides of one coin. The definition of Money of gender role is at variance with the one used in the social sciences and humanities, which defines gender role as a set of behavioral norms associated with males and with females, respectively, in a given social group or system. In this contribution I will use Money’s definition. Transsexualism can be best defined as an extreme form of gender dysphoria. Gender dysphoria is a discrepancy between gender identity/role on the one hand and the physical characteristics of the body on the other. In transsexualism, the gender identity/role of one sex coexists with the primary and secondary characteristics of the other sex in one and the same person. The current definition further requires that there is no history of a disorder of sexual differentiation. To nontranssexuals this problem is so alien and unimaginable that it is difficult to sympathize with a transsexual’s predicament. Maybe a bit of their distress becomes conceivable when a man tries to imagine what it would mean to him if he would develop breasts. This is not theoretical. It is a medical condition known as gynecomastia. Or that a woman experiences a deepening of her voice and a male type of beard and body hair growth, which is a relatively common clinical condition. Though most of the time medically insignificant, these conditions are subjectively experienced as a detriment, a forfeiture of one’s womanhood or manhood. Transsexuals live permanently in this situation of feeling that their physical body denies who they are. Transsexuals feel trapped in their bodies. With the existing techniques of assessing biological parameters of sex, on medical evaluation of transsexuals, no objective signs of intersexuality can be found. Therefore, in traditional medical practice a transsexual will be advised to undergo psychotherapy to achieve that his/her body concept, perceived as a mental function, will concur with the actual physical body. The transsexual will view such an advice as improper since it is totally at odds with how s/he perceives and knows his/her problem. The body is not “me”; the gender identity/role is the true “me”. This intimate and trusted knowledge of the self is, in fact, not different from what nontranssexuals experience in self-reflection with the cardinal difference that in their case it “happens” to agree with their physical body. Given the fact that transsexuals truthfully view their gender identity/role as correct and their body as totally wrong, psychotherapy to reconcile their gender identity to their body is doomed to fail. Transsexuals do not feel that they have the luxury to opt either for psychotherapy or for sex reassignment. In their reflections there are no options; there is only one way out of their deadlock: the “body” must follow the “mind”.

Over the last decades, there has been an advocacy of androgyny. Sex differences are conceived of as constructed or socioculturally determined. In one and the same breath, it is proposed that the need for sex reassignment of transsexuals would vanish if our society would embrace androgyny. One communication with a transsexual will reveal that this is not the case. Androgyny is a psychosocial term. It refers to psychological and social properties/attributes/options of men and women. It views the latter as unnecessarily dichotomous, and it advocates that the boundaries between the sexes stemming from this dichotomy should be abolished to allow personal growth and fulfillment. Proponents of androgyny often assume that a greater acceptance of fluidity in sex roles would resolve the transsexual’s problem. Those who treat transsexuals know that this is not the case. The transsexual’s real problem is the physical body, the flesh, that is experienced as alien, not as a part of the self, an unimaginable problem to the nontranssexual. To put it technically: transsexualism is not sex role dysphoria, it is physical body sex dysphoria.

Transsexuals themselves are not enthusiastic advocates of an androgynous life style. Living as a traditional member of the opposite sex is so highly valued that any tampering or playing with sex roles would be viewed as undermining their serious

desire and attempts to become a member of the opposite sex. In the first instance in the process of sex reassignment, most transsexuals live, therefore, conventionally male and female lives. Over time after their sex reassignment, this usually gradually resolves.

Clinicians have recognized subtypes among transsexuals. One subdivision is 'early onset transsexuals' versus 'late onset transsexuals' applying to male-to-female and female-to-male transsexuals. Early onset transsexuals, as a rule, have shown a great deal of gender nonconformity as young children and already early in life realize they are of the 'wrong biological sex' which feeling is reinforced by the hormones of puberty. These induce progressive alienation from their physical bodies as nonself. As a rule they are sexually oriented towards men. Their counterpart are the late onset transsexuals who more gradually develop gender dysphoria, not rarely after prolonged transvestic episodes, and transit to the other sex in or after their forties. Clinically, this distinction is meaningful since late onset transsexualism is more difficult to diagnose with reasonable certainty and the outcome of sex reassignment is statistically less favorable than in early onset transsexuals. Not rarely, these subjects are in the diagnostic phase sexually oriented to women. A reputable expert has labeled early onset transsexualism as homosexual transsexualism and late onset transsexualism as nonhomosexual transsexualism in males, the latter often characterized by autogynephilia (love of oneself as a woman, associated with erotical arousal by the thought or image of oneself as a woman) (Blanchard, 2005). Most transsexuals do not recognize themselves in this typology. First, the so-called homosexual male-to-female transsexuals do not view themselves as homosexual. If they interact with a man before sex reassignment surgery, they view themselves in their sexual interaction as 'women for the time being handicapped by a male anatomy', not as homosexual men interacting with another homosexual man. Their potential partners at that stage, though, are usually homosexual men who are frustrated by the denial of manhood on the side of the transsexual. Postsex reassignment surgery these transsexuals have a neovagina and their partners are heterosexual men and the sexual behavior of these transsexual subjects can no longer be labeled as homosexual. So, the label homosexual lacks permanence as a description of a subtype. The same applies to nonhomosexual transsexuals who are in the early phase usually but not always sexually oriented towards women. Postoperatively, one study showed about 50% experience a shift in sexual orientation to men (Lawrence, 2005). Preoperatively, many subjects experience sexual arousal to cross-dressing or to fantasies about themselves as women ('autogynephilia'). In all probability, with a male anatomy experienced as an alienation from the self, there are not many other options for sexual fantasies about oneself than to imagine oneself as woman. This assumption is substantiated by the observation that postreassignment surgery episodes of autogyenephilia declined from occurring in 49% preoperatively to 3% postoperatively (Lawrence, 2005). These observations argue against the use of the term nonhomosexual and autogyenephilia as a trait of late onset transsexuals since they lack permanence in the course of the lives of transsexuals.

Homosexuality

The term sexual preference is probably fundamentally incorrect. Heterosexuals are inept to fall in love and erotosexually interact with members of the same sex, and, likewise, homosexuals not with members of the opposite sex. It is rather that people are 'destined' to a sexual orientation than that they have a preference in the true sense of the word. Most people arrive in (late) teenage, but sometimes much later, at a clear self-definition of their sexual orientation, heterosexual or homosexual or in-between. In the latter case it is rarely 50%–50%. This self-definition may take some sexual experimentation. It is likely that the prevailing societal context with its social, legal and religious norms, and their internalization by subjects, play a significant role in the sometimes long searching process of one's true sexual orientation. A societal context with a hostile attitude towards homosexuality may be so intimidating to individuals that it leads to denial or reluctance to (self)-admit one's sexual orientation.

There is no convincing evidence that sexual orientation is amenable to a fundamental change, from homosexual to heterosexual.

Biomedical research into transsexualism and homosexuality

The leading principle into the biomedical research of homosexuality/transsexualism has been to identify biological female traits in male-to-female transsexuals and male homosexuals and male biological traits in female-to-male transsexuals and female homosexuals. Not stated explicitly, the paradigm in this type of research is that expressions of sexuality, such as transsexualism and homosexuality, are variations on the fundamentals of male/female dichotomy and heterosexual interaction (Gorman, 1994). Some researchers do not differentiate between homosexuality and transsexualism, viewing them as evidence of femininity in males and of masculinity in females. This approach is flawed and lacks thorough enquiry into the matter, since the two behaviors are fundamentally different. Transsexuals' deepest desire is to become members of the opposite sex and their expressions of cross-gender behavior are serious attempts to live the life of the desired sex. The desire to become a member of the opposite sex is alien to homosexuals and the effeminacy of some homosexuals is not rarely a caricature of women's manners, sometimes acted out only in the company of peers and not in other contexts. To the best of my knowledge the assumption of female traits in male homosexuals and, vice versa, of male traits in female homosexuals has been made at face value and its validity to guide biomedical research may be questioned. This issue has been reviewed recently finding large degrees of fluidity in populations and eras (Sandfort, 2005). To regard a subset of male-to-female transsexuals as feminine homosexuals (Blanchard et al., 1987) is in my view erroneous. The very definition of homosexuality states that there is mutual pleasure from having intercourse with a person with the same body/genital morphology. A preoperative transsexual having intercourse with a man could, at first sight, be

labeled as homosexual, but a major obstacle to labeling it as homosexual is that the transsexual does not derive pleasure from the arousal of the male genitalia as true homosexual men do, rather the contrary: this type of sexual encounters reaffirms the alienation from the male sexual anatomy that male-to-female transsexuals experience. In my clinical experience male-to-female transsexuals find sexual encounters with homosexual men not complementary to their sexual imagery, and vice versa.

Sexual interest in and sexual interaction with members of the opposite sex (heterosexuality) are the statistical norm, and the desire of a man for sexual interaction with another man, has been, to fit the heterosexual model, viewed as a manifestation of a female brain differentiation (and of a male brain differentiation in lesbians). For its theoretical foundation animal experimentation has been widely used, to test whether animals with a male anatomy could be hormonally manipulated to sexually interact with a male. And indeed this has been possible (Baum et al., 1990; Paredes and Baum, 1995) (see accompanying paper by Michael Baum with a review of his own and others' work). The eminent animal researcher Beach (1978) has reviewed the importance and relevance of animal models for sexology to the human species. Beach has remarked that the term homosexual in animal research has been used in two different contexts (1) as a description of individuals that exhibit a coital pattern typical of the opposite sex and (2) as a description of individuals that exhibit coital responses typical of their genetic sex but do so in response to like-sexed partner. Beach further noted that, particularly in the study of lower mammals, there is a mutually exclusive and inflexible separation of the copulatory motor patterns of males and females. Mounting and pelvic thrusting are usually viewed as exclusively male patterns, presupposing a male brain organization, whereas lordosis and reception of mounting and intromission are considered female patterns, presupposing a female brain organization. In some research male homosexual behavior is viewed as receiving intromission of one male (female brain organization?) from another male (with a male brain organization?) The alleged brain organization of the latter male is often not well defined. As an inserter this person would qualify for a male brain organization but yet must be viewed as homosexual because he interacts with another male. Suffice it to say that sexual activity of the human species (both heterosexual and homosexual) is less mechanistic and robot-like than the above described copulatory patterns. Copulatory patterns and associated eroto-sexual behavior is fluid and flexible to an extent which is not observed in animal experimentation of lower mammals but demonstrable in primates (Wallen, 2005). And more specific for homosexuals, the insertor may become the insertee and vice versa.

A remarkable finding in animal research has been that olfactory clues play a significant role in seeking out sexual partners of the opposite sex (Kelliher and Baum, 2001; Paredes and Baum, 1995). Whether these olfactory clues have a counterpart in the human with regard to partner selection has not been thoroughly investigated but is addressed by Michael Baum in his contribution. The a priori likelihood is, however, limited. Compared to most animal species, olfactory capacity of the human species is limited. Further, the present western culture makes every possible effort to eliminate body odors by frequent bathing or to override them with synthetic odors like deodorants and perfumes and the like, particularly when sexual encounters are anticipated.

Biomedical research following the above theoretization of femaleness in male homosexuals and maleness in female homosexual got a major impetus when methods for accurate measurements of androgens and estrogens, often referred to as respectively male and female hormones (semantically significant!), became more widely available. At least 20 studies have measured peripheral levels of sex steroids in male homosexuals and at least five studies did so in female homosexuals. A considerable number claimed to find differences between homosexuals and heterosexuals in the theorized direction: less 'male hormone' and/or more 'female hormone' in male homosexuals and vice versa in female homosexuals. These findings have been reviewed by several authors (Gooren, 1990; Meyer-Bahlburg, 1977, 1979) and must be dismissed as suffering from faulty design and interpretation. Moreover, with the present insights into hormonal action it is evident that a peripheral blood level of a hormone does indeed provide an indication of the strength of the signal but its eventual biological effect will be codetermined by properties of the hormone receptor and postreceptor events, rendering the peripheral blood hormone level as only one of the indices of the strength of a hormonal signal. In other words, in some men hormone blood levels may be somewhat lower but yet provide a stronger biological androgenic signal than in other men who have slightly higher hormone levels.

Capitalizing on these new insights studies have been performed to analyze whether genetic properties of sex steroid receptors in transsexuals and homosexuals differ rendering them differently sensitive to the masculinizing action of sex steroids. In a recent study sex steroid-related genes were investigated in male-to-female transsexuals who appeared to have longer estrogen receptor beta repeat polymorphisms but no differences were found with regard to the androgen receptor and the aromatase gene. Regression analysis revealed significant partial effects for all three of the above polymorphisms as well as between the androgen receptor and aromatase gene (Henningsson et al., 2005). However, another study in monozygotic and dizygotic twins could not establish any relation between properties of the androgen receptor and a rating scale measuring masculinity and femininity (Loehlin et al., 2004) which had been earlier found by Macke et al. (1993). Gene properties coding for aromatase, the enzyme which converts androgens to estrogens were not different between homosexual and heterosexual men (DuPree et al., 2004). As a clinical endocrinologist I am strongly inclined to put greater value on clinical manifestations of alleged altered hormone profiles in transsexuals/homosexuals which usually turn out to be nonexistent or very subtle, and of dubious clinical relevance. In clinical practice numerous patients are encountered with gross abnormalities of their hormonal profiles. As a rule this does not impact on their gender identity or sexual orientation. So, the a priori likelihood that very subtle changes in hormonal profiles and action, not strong enough to be clinically manifest, would impact on gender identity and/or sexual orientation is not large.

Somewhat more sophisticated, but, from an endocrine viewpoint, misguided, was the search for evidence of an estrogen-positive feedback signal on secretion of luteinizing hormone, the endocrine correlate of ovulation in women of fertile age. In lower mammals prenatal androgenization masculinizes behavior and simultaneously abolishes the capacity to display a positive estrogen feedback signal. The search for the estrogen-positive feedback signal in homosexual and transsexual males was, if it were demonstrable, thought to be proof of a less-than-normal male brain androgenization in their prenatal lives. Several studies have claimed to demonstrate an estrogen-positive feedback in homosexuals and transsexuals (Dorner, 1988; Gladue et al., 1984), but the designs of these studies were inadequate in the sense that they failed to demonstrate that all requirements of a true estrogen-positive feedback were fulfilled. Replication studies by Gooren (1986a,b) demonstrated that in transsexual and homosexual males with their normal male hormonal milieus an estrogen-positive feedback cannot be elicited. However, in transsexuals, having undergone sex reassignment and having subsequently a female hormonal milieu the estrogen-positive feedback became demonstrable. So, it appeared that rather the actual endocrine milieu, than the prenatal endocrine history, determines whether an estrogen-positive feedback can be elicited. Maybe an even more important case in point is that in primates, in contrast to lower mammals, prenatal androgen exposure of neuroendocrine structures does not abolish the capacity to respond with an estrogen-positive feedback, which fundamentally undermines the attempts to use the estrogen-positive feedback as a telltale of prenatal brain androgenization (Gooren, 1990).

So, in conclusion, attempts to find signs of a female hormonal milieu in male-to-female transsexuals and male homosexuals and vice versa have been unsuccessful. It is disconcerting that so many faulty endocrine studies passed the review process in journals which reported these findings.

An intriguing observation is the sex ratio of male-to-female transsexuals versus female-to-male transsexuals which in westernized countries is in the order of 3:1 (van Kesteren et al., 1996). In Eastern European centers a higher prevalence of female-to-male transsexualism has been noted but recent years have sometimes shown a shift to more male-to-female transsexuals; but this observation awaits a proper quantitative analysis. There is presently no plausible explanation for this discrepancy in sex ratio.

Impact of hormones on gender identity/sexual orientation. Lessons from clinical syndromes

Sexual differentiation begins with the sex difference of the chromosomes established at conception. In humans, no evidence can be found that the combination of chromosomes present in all cells (normal: XY, XX, or abnormal: XXY, XYY, XO etcetera) of the body has a direct effect on gender identity or sexual orientation. Rather, the influence is indirect and derivative through determination of the nature of the embryonic gonadal anlagen and their hormonal products (Gooren, 1990; Hughes, 2001; Migeon and Wisniewski, 2000, 2003; Money, 1981).

In the mid-1900s, it became clear from animal experimentation that the process of sexual differentiation is not completed with formation of the external genitalia but that the brain, as substrate of sexual and nonsexual behavior, also undergoes sexual differentiation to match the other characteristics of sex. In lower animals, evidence has accumulated that the same hormonal organizing principles of sexual dimorphic differentiation account for both the genitalia and the brain (Gorski, 2002; Hughes, 2001; Migeon and Wisniewski, 2000, 2003; Swaab et al., 2002). This hormonal action of testosterone has been termed organizational, and it is exerted during a rather circumscribed, so-called critical period of prenatal or early postnatal development. In some species of lower mammals part of this action, particularly the defeminization, is mediated by estrogens through aromatization of testosterone. The issue of defeminization by estrogens is addressed by Michael Baum in his twin paper in this volume and in Baum (2003) and McEwen (2001) but estrogen-driven defeminization does not occur in primates and the human, as became apparent from men who have a estrogen receptor defect or an aromatase deficiency, and, therefore, have lacked a normal estrogen stimulus prenatally or postnatally (Rochira et al., 2002).

The main regions of the mammalian brain involved in sexual differentiation are the hypothalamus, the septum, the bed nucleus of the stria terminalis, the preoptic area, and the amygdala (Gorski, 2002; McEwen, 2001; Swaab et al., 2002). The sexual dimorphic differentiation of the human brain is less well documented, but a number of sexual dimorphic nuclei have been found (sexual dimorphic nucleus (SDN) and the bed nucleus of the stria terminalis (Gorski, 2002; Swaab et al., 2002). The morphologic sex difference in the SDN is not established until the first postnatal years following a period there are no significant sex differences in circulating sex steroids; and the sex difference of the bed nucleus of the stria terminalis appears only in adulthood (Swaab et al., 2002). While the mechanism of sexual differentiation in laboratory animals is clearly orchestrated by gonadal steroids (Gorski, 2002; Swaab et al., 2002) and maybe also genetic factors (Tobet, 2002), in humans the mechanism of brain sexual dimorphism (hormonally determined or not) and the clinical relevance are not yet certain. In humans this information is to be obtained from “experiments of nature”: genetic and endocrine disorders that spontaneously occur in the fetus or result from exposure to exogenous hormone or estrogenic drugs during pregnancy (Gooren, 1990; Migeon and Wisniewski, 2000, 2003; Money, 1981). But the brains of subjects suffering from these conditions have not been studied. But overall, clinical observations support the hypothesis that in human prenatal development, sexual brain differentiation is subject to effects of androgens, but these are not of the hormonal-robot type found in subprimate mammals, in which sex steroids, in the set of behaviors studied, typically exert a simple on–off effect on sexual functioning, both in their organizational and activational effects (Gooren, 1990; Migeon and Wisniewski, 2000, 2003; Money, 1981), and there are certainly other unidentified factors that modulate or override androgen effects on the central nervous system. For instance, male and female cells differ because of differential effects of sex chromosome

genes expressed within the cells themselves (Arnold et al., 2003).

Disorders of sexual differentiation

Human sexual differentiation is a multistep, sequentially interrelated process in which genetic information is translated into the phenotype of a person who subsequently establishes a male or female identity and an awareness of sexual orientation (Gooren, 1990; Money, 1981). The human embryo is initially bipotential with respect to genital development, and consequently, disorders in any of these steps can result in ambiguity of the genitalia (Hughes, 2001; Migeon and Wisniewski, 2003), and the brain as substrate of sex typical and sexual behaviors might be bipotential in its development as well.

It is reasonable to assume that a neural substrate corresponding to traits and self-concepts of being male or female and of sexual orientation will eventually be present in a person's life. The factors which determine these self-concepts have been hotly debated over the last years, with fiercely opposing views. One school of thought was that at the time of birth there was a 'psychosexual neutrality' of children permitting, for instance, assignment of a newborn with ambiguous genitalia, regardless of the endocrine history of the newborn, to one sex or the other, on the basis of a prognosticated best future functioning as male or female. The latter based on the prognosis on the "optimal sex" for the newborn, the elements of which are an overall sex-appropriate appearance with stable gender identity, good sexual function (preferably combined with reproductive function if attainable), minimal medical procedures, and a reasonably fulfilling life hampered as little as possible by the condition (Meyer-Bahlburg, 2002; Migeon and Wisniewski, 2000, 2003). The concept of 'psychosexual neutrality at birth' has been attributed to John Money but is based on an incomplete quotation of Money's writing. Based on his extensive experience with intersexed children Money arrived at the conclusion, that more than genetic and hormonal factors, sex of assignment is a prognosticator of one future gender identity (Money, 1991, 2002). So, Money, in fact, did not exclude biological factors but was of the opinion that they were lower in the hierarchy of factors shaping one's future gender identity (Money, 1981). The opposing view is that, at the time of birth, a (biologically determined) "neural bias" is already present with regard to future gender identity/role and sexual orientation, determined by prenatal factors such as the hormonal milieu (Meyer-Bahlburg, 2002; Migeon and Wisniewski, 2000, 2003; Money, 1981) which should guide sex assignment of the newborn with ambiguous genitalia (Reiner and Gearhart, 2004; Reiner and Kropp, 2004).

As indicated earlier, brain research, mostly performed in lower mammals, demonstrates a significant role of prenatal and perinatal sex hormones in the sexual differentiation of brain and behavior (see the twin paper by Michael Baum, and other reports for an extensive review (Gorski, 2002; McEwen, 2001; Swaab et al., 2002)). Several studies provide evidence that such hormonal effects are undeniably present in humans as well, but the association is not sufficiently robust or absolute to draw firm conclusions (Gooren, 1990; Meyer-Bahlburg, 2002; Migeon and Wisniewski, 2000, 2003; Money, 1981, 2002).

The clinical syndromes that allow assessment of prenatal androgen effects on future gender identity/role, sexual orientation and other behaviors are 46,XX subjects with congenital adrenal hyperplasia (CAH) (Hrabovszky and Hutson, 2002; Meyer-Bahlburg, 2001; Meyer-Bahlburg et al., 2003; Money, 1991; Warne, 2003), 46,XY subjects with hypoandrogenism (Ahmed et al., 2000; Melo et al., 2003; Meyer-Bahlburg, 1999; Migeon et al., 2002a,b), such as complete and partial androgen insensitivity, and children with nonhormonally induced severe genital malformations such as cloacal exstrophy and penile agenesis/ablatio/micropenis who have been assigned to the female sex but whose prenatal androgen production/exposure has been similar to other males (Reiner, 2002; Reiner and Gearhart, 2004; Reiner and Kropp, 2004; Wisniewski et al., 2001).

Complete androgen insensitivity

Children afflicted with the androgen insensitivity syndrome (AIS) have a 46,XY karyotype and testes as gonads (Hines et al., 2003; Wisniewski and Migeon, 2002; Wisniewski et al., 2000). An abbreviated blind vaginal pouch is present, but no uterus or fallopian tubes. Because the external genitalia have a normal female appearance, the disorder of these patients is often unnoticed at birth. Surgical repair of an inguinal hernia containing a testis may reveal the condition. Hormonal puberty is, without intervention, feminizing due to the aromatization of endogenous androgens to estrogens. In cases of complete AIS, sex assignment and rearing are almost invariably female. The differentiation of gender identity/role is feminine (Boehmer et al., 2001; Ghali et al., 2003; Hines et al., 2003; Melo et al., 2003; Meyer-Bahlburg, 1999; Migeon et al., 2002a,b; Minto et al., 2003; Wisniewski and Migeon, 2002; Wisniewski et al., 2000). This fact is theoretically important in showing that the nature of the chromosomes and gonads per se does not dictate gender identity and role. And further, that the virtual absence of androgen exposure is associated with a female gender identity and a sexual orientation towards men. Another, theoretically important aspect of this condition is the high circulating levels of estradiol, derived from their elevated levels of testosterone production (to which these subjects are insensitive). In lower mammals prenatal estrogen exposure has behaviorally a defeminizing effect, but in the human this apparently is not the case, as is also true for nonhuman primates (Rochira et al., 2005; Wallen, 2005).

In adulthood gender identity/role and sexuality conform to typical heterosexual feminine expectations.

Partial androgen resistance syndromes

The spectrum of phenotypes in 46,XY may include individuals with almost normal female external genitalia, children with ambiguous genitalia (perineoscrotal hypospadias, a microphallus, and cryptorchidism), and a normal male phenotype (Ahmed et al., 2000; Melo et al., 2003; Meyer-Bahlburg, 1999; Migeon et al., 2002a,b). There may be some

(Ahmed et al., 2000; Ghali et al., 2003) relation between the nature of the androgen receptor defect and the phenotype. At puberty, because of the androgen insensitivity, the development of male secondary sex characteristics is not very pronounced. Gynecomastia develops usually as a result of an imbalance in androgen–estrogen action. Less severe cases may have either hypospadias or a normal male phenotype and normal male development at puberty with azoospermia.

There is considerable variability in expression of partial AIS (Ahmed et al., 2000; Boehmer et al., 2001; Melo et al., 2003; Meyer-Bahlburg, 1999; Migeon et al., 2002a,b). Minor deviations may go unnoticed or may be repaired by surgery (e.g., hypospadias). In more severe cases, the child has ambiguous genitalia. In these children the problem of sex assignment has arisen.

In practice, the chances for relatively normal development have been better in a female direction (Ahmed et al., 2000; Melo et al., 2003; Meyer-Bahlburg, 1999; Migeon et al., 2002a,b). If a male sex of rearing has been chosen, reconstructive surgery of the genitalia has usually been performed in childhood, with surgical correction of pubertal gynecomastia. The majority of 46, XY intersex patients with partial androgen insensitivity seem to develop an identity commensurate with the assigned gender and only rarely change their gender later (Meyer-Bahlburg, 2002). Childhood gender identity will in most cases continue into adolescence and adulthood, but patient-initiated gender change in intersex patients does seem to happen more often in adolescence and adulthood than in childhood. More female-assigned 46, XY patients initiate gender change to male than male-assigned 46, XY patients to female, possibly indicating the prenatal effects of androgens (Hrabovszky and Hutson, 2002; Meyer-Bahlburg, 2001, 2002). A recent review found an approximately 10% self-initiated sex reassignment (Mazur, 2005).

5α-Reductase deficiency

5α-Dihydrotestosterone (DHT), the most potent natural androgen, is formed exclusively through 5α-reduction of T by the enzyme 5α-reductase (Mendonca, 2003; Russell and Wilson, 1994). Affected people are born with labioscrotal folds and a clitoridean penis. At puberty they become moderately virilized or remain eunuchoid with enlargement of the clitoridean penis. No breast development is seen.

In the first reports it was claimed that these people were reared as girls during childhood, but after pubertal physical changes, took up life as men (Russell and Wilson, 1994). The interpretation offered was that the pubertal surge of testosterone apparently induces a reversal of gender identity and role and generates a "male sex drive" (Russell and Wilson, 1994). Later studies show that this interpretation probably needs some modification (Mendonca, 2003). Local people are usually aware of the genital disorder of these neonates and of their potential future male pubertal development. In the recent study from Brazil (Mendonca, 2003), 25 of 26 affected with 5α-reductase type 2 deficiency were assigned at birth to the female sex and raised as girls. Thirteen changed to the male sex after puberty. This was associated with some virilization of the external genitalia. There was no straightforward relationship between the severity of the condition and change of gender (Mendonca, 2003). It is of note that both testosterone itself and 5α-dihydrotestosterone are capable of masculinizing the brain in nonhuman primates (Wallen, 2005) so prenatally there has been potentially a fair amount of brain masculinization in subjects with this condition.

17β-Hydroxysteroid dehydrogenase deficiency

17β-Hydroxysteroid dehydrogenase 3 is involved in the terminal step in the synthesis of testosterone in the Leydig cell and of estradiol in the ovarian granulose cell (Andersson et al., 1996). Subjects with an XY chromosomal pattern and testes affected with 17β-hydroxysteroid dehydrogenase 3 deficiency have more or less female external genitalia due to lack of an effective androgenic stimulus at the time of the differentiation of the external genitalia (Andersson et al., 1996; Boehmer et al., 1999; Wilson, 1999). Such children are usually assigned to the female sex at birth and raised as girls (Wilson, 1999). A particular feature of this disorder is that the testosterone production increases with time (due to a higher LH drive and alternative pathways of testosterone production), and subjects may have near-normal testosterone levels at the time of puberty inducing substantial virilization. There are several reports of affected individuals raised as females who have changed their gender role behavior from female to male at the time of expected puberty (Wilson, 1999). This is not universally the case but appears to happen in approximately 50% of the reported cases in the literature (Andersson et al., 1996; Wilson, 1999). Children with 5α-reductase deficiency and 17β-hydroxysteroid dehydrogenase deficiency have genital ambiguity on the basis of deficient prenatal androgen exposure. It is the nature of these endocrine defects that biological activity of androgens at the time of puberty becomes stronger compared to prenatal life. Several studies document that approximately 50% of these children, originally assigned to the female sex, initiate a reassignment to the male sex, arguing in favor of the effects of prenatal androgen exposure on future gender identity. The latter two syndromes with a less than normal prenatal androgen exposure and a much stronger androgen exposure following puberty pose the theoretically interesting question whether androgen exposure in puberty is a contribuant to sex reassignment. So, the least one can say is that prenatal androgen exposure is associated with an increased chance of later patient-initiated gender reassignment to male after initial female assignment in infancy or early childhood (Meyer-Bahlburg, 2005).

Congenital adrenal (virilizing) hyperplasia

Congenital adrenal hyperplasia (CAH) is a disorder occurring in both sexes involving undue/untimely exposure to androgens. Early reports indicated an overriding influence of the sex of assignment and rearing on the gender identity of CAH girls (Money, 1981, 1991). If CAH subjects were assigned as girls, they turned out to have a female gender identity, but with tomboyish behavior in play and activity and high energy expenditure—a marked masculine shift on the scale of sex dimorphic behavior likely due to prenatal and possibly postnatal

androgen exposure (Meyer-Bahlburg, 2001; Meyer-Bahlburg et al., 2003; Money, 1991).

These observations also extend to childhood and later indices of maternal interest. This is more true for the salt wasting form of CAH than for the simple virilizing form who show a large variability in masculinized gender role behavior, though this is intra-personally often compatible with a core female gender identity (Meyer-Bahlburg et al., 2003). Some are less contented with life as women without having an explicit gender identity disorder (Meyer-Bahlburg, 2001; Meyer-Bahlburg et al., 2003). In a report of older CAH subjects reared as girls, 37% rated themselves as homosexual or bisexual or they had fewer heterosexual experiences than the comparison group (Meyer-Bahlburg, 2001). This finding has been further corroborated (Meyer-Bahlburg et al., 2003). Another study was less affirmative in this regard (Money, 1991). Nevertheless, retrospective studies indicate that that there may be a decreased sexual interest and below-average engagement in heterosexual relationships. This may also be due to anatomical inadequacies of the genitalia (Meyer-Bahlburg, 2001; Meyer-Bahlburg et al., 2003; Warne, 2003). Further, hirsutism may be a confounding factor. The likelihood of a gender change later in life in such females correlates with the presumed degree of prenatal androgen exposure, though the association is not very strong. Naturally, the degree of prenatal and postnatal androgen exposure also determines the extent of genital ambiguity, which, together with the postnatal biography and considerations of quality of life, may also be factors in a change of gender. Predictors of gender change are stigmatization, gonadectomy and/or feminizing surgery after the age of 3 years. A relative absence of gender dysphoria in childhood does not preclude a gender change later in life.

By contrast, those subjects assigned as boys due to the high degree of masculinization of their external genitalia successfully developed a male gender identity and role (Money, 1991) though patient-initiated reassignments to the female gender have been reported (Meyer-Bahlburg, 2001; Meyer-Bahlburg et al., 2003).

Prenatal dexamethasone treatment of pregnant mothers possibly bearing a child affected with CAH has become an option, though it is still experimental (New et al., 2001, 2003). Treatment must start "blindly" by or before the sixth or seventh postmenstrual week until the diagnosis can be made by chorionic villous biopsy at week 10 or 11 or by amniocentesis at weeks 14 to 16 (Forest and Dorr, 2003; New et al., 2003). The treatment requires intensive guidance of the patient but is efficacious (Forest and Dorr, 2003).

Prenatal exposure to exogenous hormones

Estrogens or estrogenic drugs (predominantly diethylstilbestrol (DES) and progestins were administered to pregnant women, notably between 1940 and 1970. Synthetic progestins have, depending on their chemical formulas, antiandrogenic or weak androgenic biologic activity. In male fetuses prenatal exposure to progestins and/or estrogens may have suppressed their endogenous testosterone production by the powerful negative feedback action on the hypothalamic–pituitary–testicular axis (Gooren, 1990). Prenatal exposure of female fetuses to DES or progestins has not impaired their subsequent self-identification as female (Money and Mathews, 1982), but a higher incidence of homosexuality or bisexuality (25%) in adulthood has been reported in a sample of such women (Ehrhardt et al., 1985). Several follow-up studies of prenatal exposure to DES and/or progestin in men have indeed found them to display a degree of nonconformity in stereotyped gender behavior, but a clear-cut effect on sexual orientation or gender identity has not been established (Gooren, 1990).

Boys with malformations of the genitalia or extreme micropenis

Boys born with a cloacal exstrophy have normal testes and a presumed normal prenatal exposure to androgens. About 50% of those assigned to the female sex are reported to evidence dissatisfaction with this and change to life as a male (Reiner, 2002; Reiner and Gearhart, 2004; Reiner and Kropp, 2004). The interpretation is that prenatal androgen exposure underlies this change and is consistent with the notion that there is an increased risk of later patient-initiated gender reassignment to male after female assignment in infancy or early childhood in 46,XY subjects born with ambiguous genitalia with other etiologies (Meyer-Bahlburg, 2005). The latter plays probably also a significant role in the change of gender observed in boys with cloacal exstrophy. It is clear that androgen exposure in XY subjects predisposes but does not assure a male gender identity. If the newborn has originally been assigned to the female sex, there is an approximately 40–50% chance of development of a female gender identity.

More or less in contrast, in a series of 18 children born with a micropenis, 13 assigned as males and 5 as females, all subjects were satisfied with their sex of rearing in adulthood, though both men and women expressed dissatisfaction with their genital status (Wisniewski et al., 2001).

Summary of the findings

In summary, the evidence available to date permits the following conclusions: (1) the organizational effects of prenatal androgens are more noticeable in gender role behavior than in gender identity; (2) gender identity can develop as female or male over wide variations of gender role behavior; (3) there is suggestive, but not conclusive, evidence that a male gender identity/role is more frequent in patients with a history of fully male-typical prenatal androgenization, such as in cloacal exstrophy.

While it is a legitimate scientific endeavor to research the impact of sex steroids on gender identity and sexual orientation as entities, it is appropriate to take into consideration the predicaments of the studied subjects. Having suffered from sex steroid deficiencies or other ailments of sexual differentiation, the development of their genitalia has usually not been normal which negatively affects their self image as men or women and hinders their normal psychosexual development and makes encounters with (potential) sexual partners embarrassing. It is difficult to overrate their suffering from having a not normal sexual differentiation of the genitalia. The public expression of

their gender is often codetermined by practical every-day-life considerations (passing better as one sex than the other, prevailing social climate as to the position of men and women, etcetera).

The above examples discussed gender identity and sexual orientation in subjects with a nonnormal sexual differentiation, hormonally or otherwise. These conditions are relatively rare. Traditionally, in this type of reviews no place is given to the conditions of transsexualism and homosexuality. The latter is rather common and both conditions are characterized by a normal prenatal/postnatal endocrine history. Studies to the contrary do not provide sufficiently solid evidence to be credible. In my view, these conditions provide compelling evidence that female gender identity can develop in a person with normal prenatal and postnatal androgen exposure and vice versa. Similarly, sexual attraction to women can develop with an average female (almost nonexistent) androgen exposure, while attraction to men can develop in subjects having had a normal male androgen exposure in their prenatal and postnatal lives. So while the histories of persons with a abnormal sexual differentiation undeniably point to an effect of androgens, there are codeterminants of gender identity and sexual orientation with the power of overriding effects of androgens on the brains (male transsexuals/homosexuals) or making androgen effects on the brain redundant (female transsexuals/homosexuals). These factors elude us presently and leave us puzzled about phenomena as transsexualism and homosexuality.

Results of brain morphological studies

Homosexuality

The human hypothalamus is involved in a wide range of functions in the developing, adult and aging subject and is responsible for a large number of symptoms of neuroendocrine, neurological and psychiatric diseases. Several studies have addressed sex differences in hypothalamic structures and have tried to relate morphological findings to sexual orientation.

The volumes of four cell groups in this region (interstitial nuclei of the anterior hypothalamus (INAH) 1, 2, 3, and 4) have been studied. No sex differences were found between the groups in the volumes of INAH 1, 2, or 4.In the study of LeVay (1991), INAH 3 was more than twice as large in heterosexual men as in women. It was also, however, more than twice as large in the heterosexual men as in the homosexual men, implying that part of the sexual differentiation of the hypothalamus in homosexual is in a female direction (LeVay, 1991). But later studies have been unable to confirm this finding (Byne et al., 2001). Swaab and coworkers found a cluster of cells in the preoptic area of the human hypothalamus that contains about twice as many cells in young adult men as in women, and called this cluster the sexually dimorphic nucleus (SDN) (Swaab et al., 2002). The magnitude of the sex difference in the SDN depends on age. At birth the SDN contains only some 20% of the cells found at 2 to 4 years of age. The cell number rapidly increases in boys and girls at the same rate until 2 to 4 years of age. After that age period, a decrease in cell number takes place in girls, but not in boys. Remarkably, the sexual differentiation of the SDN occurs in postnatal life, when there are no significant differences is secretions of sex steroids between girls and boys. Swaab and coworkers did not find that the SDN has different properties in homosexual men compared to heterosexual men (Swaab et al., 2001). Another morphological difference in homosexual men compared to heterosexuals was the mid-sagittal plane of the anterior commissure, in homosexual men was 18% larger than in heterosexual women and 34% larger than in heterosexual men, supposed underlying differences in cognitive function and cerebral lateralization between homosexual men, heterosexual men, and heterosexual women (Allen and Gorski, 1992) but not confirmed in a later replication study (Lasco et al., 2002). The suprachiasmatic nucleus (SCN) is the hypothalamic biological clock (Kalsbeek and Buijs, 2002). Environmental light serves as the main "Zeitgeber" to entrain the clock, which receives its input via the retinohypothalamic tract. In turn, the SCN and its projections communicate through synaptic pathways with various effector systems. These areas are known to be involved in the autonomic regulation of body temperature, blood pressure, sleep, arousal, energy metabolism, stress (HPA axis), thyroid hormone (HPT axis) and growth hormone regulation and in the regulation of the reproductive gonadal (HPG)axis, via the cyclic release of gonadotropin-releasing hormone (Kalsbeek and Buijs, 2002). Morphometric analysis of the human hypothalamus revealed that the volume of the suprachiasmatic nucleus (SCN) in homosexual men is 1.7 times as large as that of a reference group of male subjects and contains 2.1 times as many cells (Swaab and Hofman, 1990). During development, the SCN volume and cell counts reach peak values around 13–16 months after birth. At this age the SCN contains about the same number of cells as the SCN of adult male homosexuals, whereas in the reference group of male subjects the cell numbers subsequently decline to the adult value, which is about 35% of the peak value.

This study has not been replicated and it is difficult to provide a meaningful interpretation to this finding in the context of human homosexuality.

Transsexualism

The human bed nucleus of the stria terminalis (BSTc) is sexually dimorphic in size and neuron number (Chung et al., 2002; Kruijver et al., 2000; Zhou et al., 1995). Both measures are larger in males. No relationship between these BSTc measures and sexual orientation has been found whereas a striking relationship with gender identity was observed (Kruijver et al., 2000; Zhou et al., 1995). Male-to-female transsexuals had, regardless of sexual orientation or their adult endocrine status, a BSTc with a size and neuron numbers found in females. Interestingly, a male type development pattern was found in the only available brain so far of a female-to-male transsexual (Kruijver et al., 2000). The functional implications of these sex dimorphic findings are still far from clear, but it is of note that in animals subdivisions of the BST have been implicated in the regulation of, e.g., female reproductive (lordosis) and maternal behavior. The BSTc expresses both

androgen, estrogen and progesterone receptors (PRs) in the adult) and developing BSTc (Swaab et al., 2003). Alterations in hormone levels in adulthood appeared to have no effect on the size and cell number of the BSTc (Kruijver et al., 2000; Zhou et al., 1995). These findings support a concept that transsexualism is a sexual differentiation disorder of the sex dimorphic brain. There are some difficulties with this hypothesis since in a neurodevelopmental study by Chung et al. (2002) it was found that the *volume* (neuron numbers were not counted during development) of the BSTc area becomes only sexually dimorphic around the beginning of adulthood. It remains to be answered how this finding can be reconciled with the very common finding of the start of gender dysphoria early in childhood, unless the developmental course of the BSTc would have been preprogrammed already much earlier, for instance by the pre- and/or perinatal testosterone surges of which the effects only appear in adulthood. A fundamental problem with regard to these speculations is that so far there is no evidence of prenatal/perinatal/postnatal hormonal disturbance in transsexuals.

The fraternal birth order in males

In diverse samples and independent replications, homosexual men are found to have a greater number of older brothers than heterosexual men (Blanchard and Bogaert, 1997, 2004; Blanchard and Ellis, 2001; Blanchard et al., 2006; Bogaert, 1998; Purcell et al., 2000). It has been estimated that each older brother increases the relative risk of being a homosexual man by 33–48%, although these odds translate into population probability estimates of only a few percent (Blanchard and Bogaert, 2004; Blanchard et al., 2006). So, it certainly does not provide a universal hypothesis for the origins of homosexuality since the majority of homosexual men do not have this history and do not fit in this model. The hypothesis advanced in the above studies is that the late birth order, with more male siblings born earlier, could lead to a progressive immune response of the mother to androgens and/or Y-linked minor histocompatibility (H–Y) antigens which, by maternal transfer of these immune antibodies to the fetus, could impair brain masculinization of the fetus (Blanchard and Bogaert, 2004; Blanchard et al., 2006). However, why this mechanism would selectively impair only certain androgen-dependent processes, such as the brain programming, and not others, like formation of the genitalia, is not explained by this hypothesis, and not even addressed by the proponents (Gooren and Kruijver, 2002). Nor does this theory explain why the majority of boys late in birth order do not become homosexual, even if their elder brother is homosexual. To the best of my knowledge it has not been attempted to demonstrate antibodies against testosterone or proteins encoded on the Y-chromosome in women who have one or more homosexual sons with a late birth order. To the best of my knowledge, there is no known clinical syndrome that is based on antibodies to testosterone or proteins encoded on the Y-chromosome. The formation of antibodies against a steroid hormone is an unlikely event. So, while the statistical association is now well documented, the advanced hormonal explanation for the association lacks any experimental support. The advanced explanation should also be put in the light of the findings relating (lack of) androgen exposure to future gender identity/sexual orientation development which are not straightforward at all.

Digit ratios as marker of prenatal testosterone

The ratio of the second-to-fourth finger length was first proposed as a marker for prenatal androgen action in 1998, and over 100 studies have been published testing the association between the digit ratio and prenatal androgens, or employed digit ratios as a marker to investigate the association between prenatal androgens and a variety of outcomes, including behavior, fertility, and disease risks. The validity of digit ratios to serve as an adult marker of prenatal androgen action remains controversial (for review, see McIntyre, 2006). In short: adult men have longer ring fingers (fourth digits) than adult women relative to the lengths of other fingers (McIntyre, 2006). Most researchers have focused on the digit ratio of 2D:4D, the index and ring finger respectively. In adults, the point-biserial correlation between sex and right-hand, skin-surface 2D:4D is not high, even in racially homogenous samples ($r = 0.22$, ~60% overlap between male and female distributions (Manning et al., 1998). Furthermore, the large population and racial differences observed in both adults and children may introduce serious confounding (McIntyre, 2006).

Congenital adrenal hyperplasia (CAH) treated soon after birth has been the gold-standard method for studying effects of prenatal or perinatal androgens. One study found that 13 females and 7 males with postnatally treated CAH had lower 2D:4D (but only on the right hands of females and left hands of males) than 44 female and 28 male relatives unaffected by CAH (Brown et al., 2002). Another study found that 27 females and 9 males with postnatally treated CAH had lower 2D:4D (on both hands of females and only the right hands of males) than 52 female and 52 male age-matched controls unaffected by CAH (Okten et al., 2002). However, a study employing 2D:4D measured on radiographic films of the left hand failed to find a significant difference between 66 CAH females and 69 age-matched control females and 77 males, though 2D:4D was intermediate between means for unaffected males and females (Buck et al., 2003). The least one can say is that the 2D:4D ratio is not a robust marker of prenatal androgen exposure. It is also of note that there is a multitude of factors influencing bone growth prenatally. Recent endocrine insights are that that estrogens (derived from androgens) are probably more significant that androgens themselves in bone development of men (Vanderschueren et al., 2004). Several studies have attempted to link sexual orientation to 2D:4D ratio. Some established a statistical correlation between a higher 2D:4D ratio and homosexuality in men (Robinson and Manning, 2000; Williams et al., 2000), but others failed to confirm this (Voracek et al., 2005). In a sample of 'not-strictly heterosexual' women no difference in 2D:4D ratio with 'strictly heterosexual' women was found (van Anders and Hampson, 2005).

Family studies and genetics

Genetic and epigenetic factors, in particular in as far they relate to hormonal actions, have also been implicated. Sexual orientation is influenced by a number of genetic factors as appears from studies in families and twins, and from molecular genetics (Bailey and Pillard, 1991; Hamer et al., 1993; Hu et al., 1995; Pillard and Bailey, 1998; Pillard and Weinrich, 1986). Homosexual men have more homosexual brothers and homosexual women have more homosexual sisters as compared to respectively heterosexual men or women. Twin studies also suggest that this familial concentration is, at least, partly genetic. Monozygotic twins show around 30–60% greater concordance for homosexuality than dizygotic twins. Hamer and colleagues found linkage between DNA markers on the X-chromosome and male sexual orientation. In an attempt to find an explanation for the finding that male homosexual often had a maternal homosexual uncle, Hamer et al. reported a genetic linkage between the microsatellite markers on the X chromosome, i.e., Xq28, in the families of homosexual males but not for the families of homosexual females (Hamer et al., 1993; Hu et al., 1995). A recent study found extreme skewing of X chromosome inactivation in mothers of homosexual men (13% versus 4% in mothers who had no homosexual men, increasing to 23% if mothers had two homosexual sons (Bocklandt et al., 2006)). However, Rice et al. (1999) found an absence of linkage to microsatellite markers at Xq28 (Mustanski et al., 2005).

Genes are a segment of a DNA molecule that contains all the information required for synthesis of a product (polypeptide chain or RNA molecule); it is a long way to comprehend how genetic information is related to a behavioral trait such as homosexuality.

Conclusions

It is a daunting task to comment on the biological underpinnings of such complex phenomena as gender identity and sexual orientation in humans. I am not saying that human nature is not rooted in biology, obviously it is. But it is probably fair to say that we are far away from an understanding how gender identity and sexual orientation come about in the human species.

Most attempts to find a biological underpinnings have investigated effects of sex steroids, so pivotal in the differentiation of the genitalia (with strong parallels in animals and the human) and of the brain in animals. Sexology is often presented as a multidisciplinary science but the scientific rigor of studying biological underpinnings leaves much to be desired. The lightheartedness of using certain biological markers in adulthood as indicators of prenatal androgen exposure is not warranted. The conclusion is warranted that prenatal androgenization predisposes to a male gender identity development, but it is apparently not decisive. Brain studies in homosexuals and transsexuals are few and results have not held up in replication studies (the interstitial nuclei if the anterior hypothalamus in homosexuals) or are in need of replication (the nucleus suprachiasmaticus in homosexual men and the bed nucleus of the stria terminalis in transsexuals).

Genetic studies provide indications of familial clustering of homosexuality but in many homosexuals these genetic patterns cannot be recognized. The same applies to the fraternal birth order hypothesis which has a strong statistical backing but cannot be generalized to all homosexual men. The biological explanation advanced for the fraternal birth order hypothesis lacks any experimental support.

My final analysis is that the available evidence, accumulated over the past 30 years, supports a role for testosterone in the development of gender identity and sexual orientation in the human species. A role for estradiol has not been convincingly demonstrated.

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