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Sexual Hormones and the Brain: An Essential Alliance for Sexual Identity and Sexual Orientation

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Abstract

The fetal brain develops during the intrauterine period in the male direction through a direct action of testosterone on the developing nerve cells, or in the female direction through the absence of this hormone surge. In this way, our gender identity (the conviction of belonging to the male or female gender) and sexual orientation are programmed or organized into our brain structures when we are still in the womb. However, since sexual differentiation of the genitals takes place in the first two months of pregnancy and sexual differentiation of the brain starts in the second half of pregnancy, these two processes can be influenced independently, which may result in extreme cases in transsexuality. This also means that in the event of ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain. There is no indication that social environment after birth has an effect on gender identity or sexual orientation.

Sex Differences in Cognition and Aggression: Little Effect of the Social Environment

Boys and girls behave in different ways and one of the stereotypical behavioral differences between them, that has often been said to be forced upon them by upbringing and social environment, is their behavior in play. Boys prefer to play with cars and balls, whereas girls prefer dolls. This sex difference in toy preference is present very early in life (3–8 months of age) [1]. The idea that it is not society that forces these choices upon children but a sex difference in the early development of their brains and behavior is also supported by monkey behavioral studies. Alexander and Hines [2], who offered dolls, toy cars and balls to green Vervet monkeys found the female monkeys consistently chose the dolls and examined these ano-genitally, whereas the male monkeys were more interested in playing with the toy cars and with the ball. 'Neutral'

toys, such as a picture book and a toy dog, did not show sex differences in either humans or monkeys. A similar result was reported in rhesus monkeys, showing that toy preference can develop without explicit gender socialization [3–5]. Testosterone levels during pregnancy plays a role in this, because girls who are exposed to high levels of testosterone in the womb in the case of congenital adrenal hyperplasia (CAH), tend to choose boys as playmates, play preferentially with boys' toys, are generally wilder, present less interest in infants than other girls and are called tomboys [6, 7]. In addition they have some male-typical direction personality features [7]. It thus seems that these sex differences are originated early on in our evolution, before the hominids, and that they are imprinted during intrauterine development under the influence of testosterone [8] and its receptor [9]. It should be noted that children's toy preferences are not necessary predicting an adult gender identity disorder [10].

A similar conclusion can be inferred from the sex differences in spontaneous drawings. Japanese research shows that subject matter, choice of color and composition of drawings by boys and girls show clear sex differences, influenced by the hormones to which the child's brain was exposed in the womb. Girls tend to draw human figures, mainly girls and women, flowers and butterflies. Boys, however, prefer to draw more technical objects, weapons and fighting, and means of transport, such as cars, trains and airplanes, in birds-eye view compositions. Drawings by girls exposed to too high testosterone levels in the womb due to CAH begin to show male characteristics some 5–6 years later, even when treated immediately after birth [11]. Apparently, exposure to higher levels of male hormones has important and lasting effects on behavior and artistic pattern expression. Aggressive behavior in men has been related as well with prenatal testosterone levels [12], although those levels can be variable postnatally depending on the time of the day, seasonal changes and other tonic circadian rhythms [13] such as an aggressive stimuli in men [12] and sexual behavior in both sexes [14].

Organizational and Activational Effects of Sex Hormones

The fetal testicles and ovaries develop in the sixth week of pregnancy. This occurs under the influence of a cascade of genes, starting with the sex-determining gene on the Y chromosome (*SRY*). The production of testosterone and the peripheral conversion of testosterone into dihydrotestosterone between weeks 6 and 12 of pregnancy are essential for the formation of a boy's penis, prostate and scrotum. Instead, the development of the female sexual organs in the womb is based primarily on the absence of androgens [15].

Once the differentiation of the sexual organs into male or female is settled, the next thing that is differentiated is the brain, under the influence, mainly, of sex hormones such as testosterone, estrogen and progesterone on the developing brain cells and under the presence of different genes as well [15]. The changes brought about in this

stage are permanent. Later, during puberty, the brain circuits that were organized in the womb are activated by sex hormones. There are at present many additional candidate genes for a role in sexual differentiation of the brain without the involvement of hormones, since it has been found that 50 genes are expressed at different levels in the brains of male and female mouse fetuses, even before the hormones come into play [16]. Thus, sexual differentiation of the brain is not caused by hormones alone, even though they are very important for gender identity and sexual orientation.

There are two critical periods in human development where testosterone levels are known to be higher in boys: the first surge occurs during mid-pregnancy, when testosterone levels peak in the fetal serum between weeks 12 and 18 of pregnancy and in weeks 34–41 of pregnancy the testosterone levels of boys are ten times higher than those of girls [15]. The second surge takes place in the first 3 months after birth. At the end of pregnancy, when the α-fetoprotein level declines, the fetus is more exposed to estrogens from the placenta, this exposure inhibiting the hypothalamus-hypophysial-gonadal axis of the developing child. The testosterone level in boys at this time is as high as it will be in adulthood, although a large part of the hormone circulates bound. Also at this time the testosterone level is higher in boys than in girls. During these two periods, therefore, girls do not show high levels of testosterone. These fetal and neonatal peaks of testosterone, together with the functional steroid receptor activity, are thought to fix the development of structures and circuits in the brain for the rest of a boy's life (producing 'programming' or 'organizing' effects). Later, the rising hormone levels that occur during puberty 'activate' circuits and behavioral patterns that were built during development, in a masculinized and de-feminized direction for male brains or in a feminized and de-masculinized direction for female brains.

As sexual differentiation of the genitals takes places much earlier in development (i.e. in the first 2 months of pregnancy) than sexual differentiation of the brain, which starts in the second half of pregnancy and becomes overt upon reaching adulthood, these two processes may be influenced independently of each other. In rare cases, this may result in transsexuality, i.e. people with male sexual organs who feel female or vice versa. It also means that in the event of an ambiguous sex at birth, the degree of masculinization of the genitals may not always reflect the degree of masculinization of the brain [15, 17]. In addition, gender identity may be determined by prenatal hormonal influences, even though the prenatal hormonal milieu might be inadequate for full genital differentiation [15].

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

Programmed Gender Identity Is Irreversible

The irreversibility of programmed gender identity is clearly illustrated by the sad story of the John-Joan-John case (i.e. the case of David Reimer). In the 1960s and 1970s, in the context of the Behaviorism, it was postulated that a child is born as a *tabula rasa* and is subsequently forced in the male or female direction by society's conventions. Although it is true that, by the age of 2–3 years, children during preschool year are able to correctly label themselves and others according to gender [18], there is no evidence that external or social events might modify these processes. However, J. Money argued that: 'Gender identity is sufficiently incompletely differentiated at birth as to permit successful assignment of a genetic male as a girl. Gender identity then differentiates in keeping with the experiences of rearing' [19]. This view had devastating results in the John-Joan-John case (Colapinto). Money maintained that gender imprinting does not start until the age of 1 year, and that its development is well advanced by the age of 3–4 years [20]. This was, indeed, the basis for the decision to make a girl out of an 8-month-old boy who lost his penis due to a mistake during minor surgery (i.e. an operation to correct phimosis). The testicles of this child were removed before he reached the age of 17 months in order to facilitate feminization. The child was dressed in girl's clothes, received psychological counseling and was given estrogens in puberty. According to Money, this child developed as a normal female. However, Milton Diamond later made it clear that this had not been the case at all. In adulthood, this child changed back to male, married, and adopted several children [21]. Unfortunately, John had a troubled life and committed suicide in 2004. This story illustrates the enormous programming influence of the intrauterine period on gender. Other cases have been described in the literature due to enzymatic disorders or to cloacal exstrophy that support the existence of early permanent programming of brain sex by biological factors and androgen exposure, rather than by social environment and learning [for revision, see 15, 17].

Neurobiological Factors of Sexual Differentiation of the Brain

In humans, the main mechanism responsible of sexual identity and orientation involves a direct effect of testosterone on the developing brain. Complete androgen insensitivity syndrome is caused by different mutations in the gene for the androgen receptor (AR). Despite their genetic (XY) masculinity, affected individuals with complete androgen insensitivity develop as phenotypical women and experience 'heterosexual' sexual orientation, fantasies and experiences, without gender problems [22]. Partial androgen insensitivity (different locus mutations in the AR) can, however, lead to dissatisfaction with the assigned female sex [23].

On the other hand, when a male fetus has a 5α-reductase-2 or 17β-hydroxysteroid dehydrogenase-3 deficiency preventing peripheral testosterone from being

transformed into dihydrotestosterone, a 'girl' with a large clitoris is born. These children are generally raised as girls. However, when testosterone production increases in these XY children during puberty, this 'clitoris' grows to penis size, the testicles descend, and the child's build begins to masculinize and become muscular. Despite the fact that these children are initially raised as girls, the majority (60%) change into heterosexual males [15, 17], apparently due to the organizing effect of testosterone on early brain development and the activational testosterone production in puberty. Boys who are born with a cloacal exstrophy – i.e. with bladder exstrophy and a partly or wholly absent penis – are usually changed into girls immediately after birth. A survey showed that in adulthood only 65% of these children who were changed into girls continued to live as girls, and when individuals with gender dysphoria were excluded the figure dropped to 47% [24, 25]. From these examples it appears that the direct action of testosterone on the developing brain in boys and the lack of such action on the developing brain in girls are crucial factors in the development of male and female gender identity and sexual orientation, although other sexually dimorphic functions still need to be investigated.

Sex Differences in the Human Brain

A sex difference in brain weight is already present in children from the age of 2 years and sex differences can thus be expected throughout the brain from early in development onwards. In the adult human brain structural sex differences can be found from the macroscopic level down to the ultramicroscopic level. Functionally, too, a large number of sex differences in different brain regions have recently been described. Although a greater intrasex phenotype variability in males have been found for cognitive abilities, sexual differentiation of the human brain is also expressed in behavioral differences [17, 26].

When observed by our group, the structural difference in the intermediate nucleus of the human hypothalamus (InM) [27] was at first termed 'the sexually dimorphic nucleus of the preoptic area (SDN-POA)' [28]. We found this nucleus to be 2.5 times larger in men than in women and to contain 2.2 times as many cells [28]. The sex difference develops only after the age of 5 years and disappears temporarily after the age of 50 years [28–30]. Allen et al. [31] described four interstitial nuclei of the anterior hypothalamus (INAH1–4) and found, in men compared to women, a larger volume of the INAH3 and INAH2 subdivisions (respectively 2.8 and 2 times greater).

We recently localized and delineated the uncinate nucleus (Un). We found a sex differences in volume and neuron number in the INAH3 subdivision [32] (fig. 1), confirming previously reported data [33–35].

Other sex differences that could be related to sex differences in cognitive abilities have been found in the human anterior commissure, the interthalamic adhesion and in the corpora mamillaria [36].

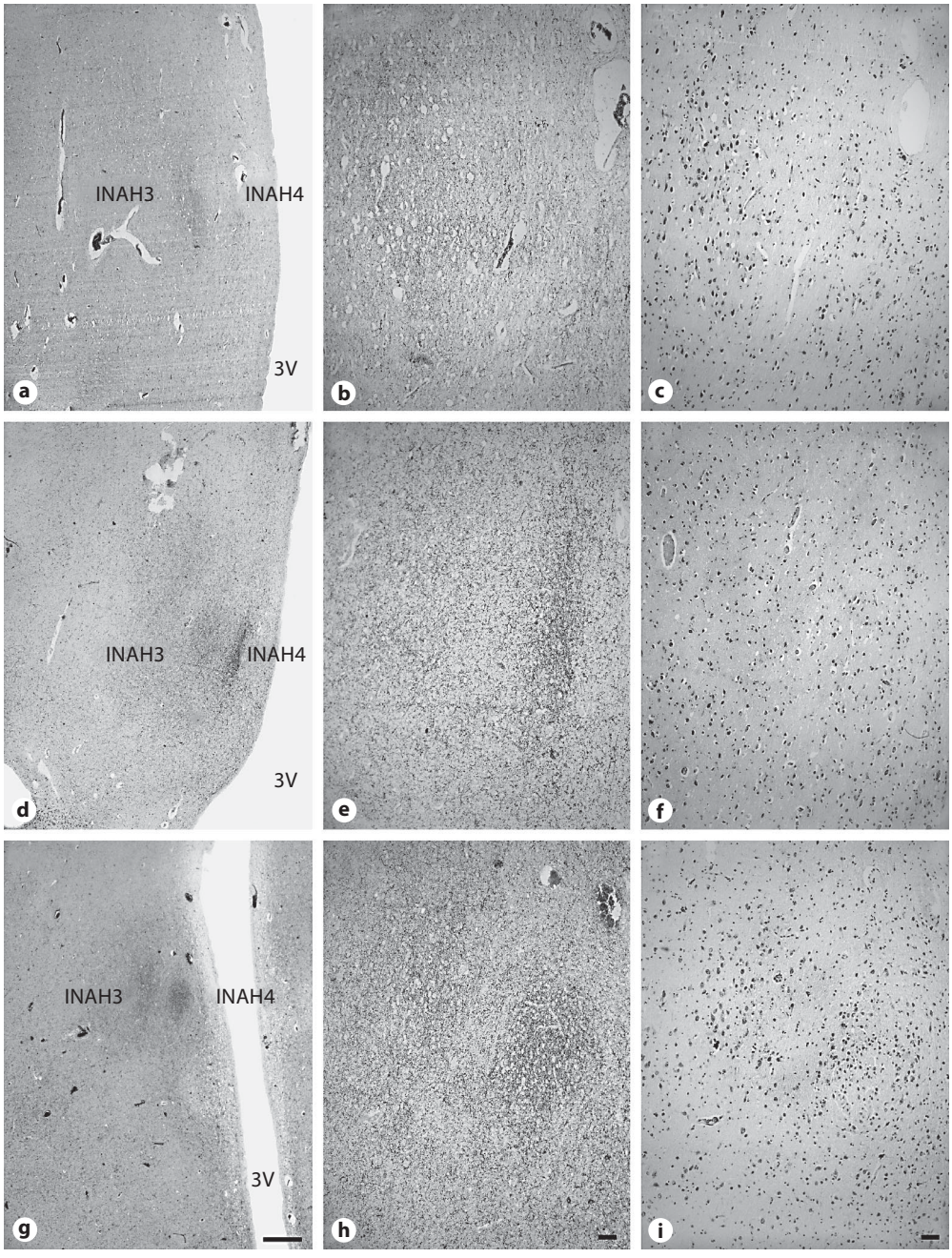

Fig. 1. Representative photomicrographs of the uncinate nucleus in man, woman and transsexual person through consecutive sections (**a–c** subject NBB # 00131; male 25 years old; **d–f** subject NBB # 01011; female 46 years old; **g–i** subject NBB # 84037; transsexual male-to-female 44 years old). **a**, **d, g** Low magnification power of the immunocytochemical stainings of Neuropeptide-Y (NPY). INAH3 and 4: interstitial nucleus of the anterior hypothalamus 3 and 4, 3V: third ventricle. Scale bar = 500 µm. **b, e, h** Details of the innervation by NPY fibers. **c, f, i** Details of the thionin consectutive staining sections. Scale bar = 63 µm. Note that the male group shows a larger number of cells in INAH3 subdivision than the transsexual and female subjetcs (**c, f. i**). From Garcia-Falgueras and Swaab [32] fig. 8, with permission.

Transsexuality

Transsexuality is the most extreme gender-identity disorder (GID) and consists of the unshakable conviction of belonging to the opposite sex, leading to a request for sex-reassignment surgery and hormonal treatment [37]. There is a vast array of factors that may lead to gender problems [for refs, see 17]. Twin and family research has shown that genetic factors play a part. Rare chromosomal abnormalities may lead to transsexuality, and it was recently found that polymorphisms of the genes for ERα and ERβ, AR repeat length polymorphism, and polymorphisms in the aromatase or CYP17 gene also produced an increased risk. Abnormal hormone levels during early development may play a role, as girls with congenital adrenal hyperplasia (CAH), who has been exposed to extreme levels of testosterone in utero, have an increased chance becoming transsexual. Although the likelihood of transsexuality developing in such cases is 300–1,000 higher than normal, the risk for transsexuality in CAH is still only 1–3%, whereas the probability of serious gender problems is 5.2%. The consensus is, therefore, that girls with CAH should be raised as girls, even when they are masculinized. Epileptic women who were given phenobarbital or diphantoin during pregnancy also have an increased risk of giving birth to a transsexual child. Both these substances change the metabolism of the sex hormones and can act on the sexual differentiation of the child's brain. There are no indications that postnatal social factors could be responsible for the occurrence of transsexuality.

Only in 23% of cases does a childhood gender problem lead to transsexuality in adulthood. With regard to sexual orientation, the most likely outcome of childhood gender identity disorder is homosexuality or bisexuality.

Transsexuality and the Brain

The theory on the origins of transsexuality is based on the fact that the differentiation of sexual organs takes place during the first couple of months of pregnancy, before the sexual differentiation of the brain. As these two processes have different timetables, it is possible, in principle, that they take different routes under the influence of different factors. If this is the case, one might expect to find, in transsexuals, female structures in a male brain and vice versa, and indeed, we did find such reversals in the central nucleus of the BSTc and in the INAH3, two brain structures that, in rats, are involved in many aspects of sexual behavior.

In men the BSTc volume was twice as large as in women and contained twice as many somatostatin neurons [38, 39]. The same was true for the INAH3, which was found to be 1.9 times larger in men than in women and to contain 2.3 as many neurons [32] (fig. 1, 2). It is remarkable that, even although a significant difference was present in total brain between man and woman ($p < 0.001$) no sex differences structural or functional were found in the INAH4 subdivision of the uncinate nucleus [32].

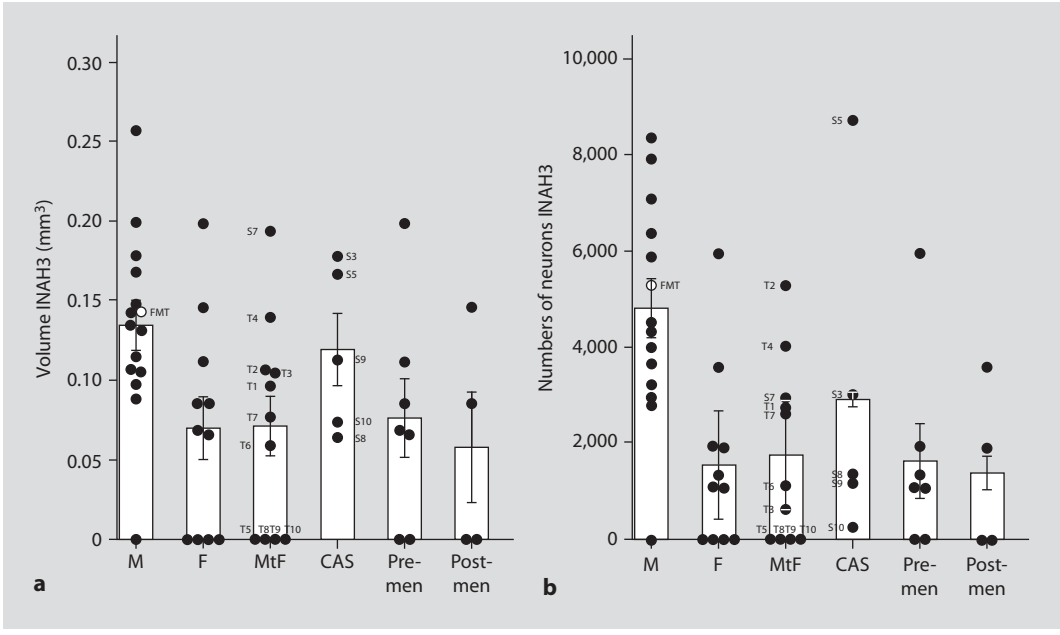

Fig. 2. **a** INAH3 volume in thionin staining in different groups, according to their gender identity and hormonal changes in adulthood. (M) control male group, (F) control female group, (MtF) male to female transsexual group, (CAS) castrated male group, (PreM) premenopausal women, (PostM) postmenopausal women. Bars represent means and standard errors of the mean (SEM). MtF and F groups were statistically different compared to the M group ($p < 0.018$ and $p < 0.013$, respectively). Hormonal changes in adulthood (CAS vs. M and PreM vs. PostM groups) showed no differences in INAH3 volume. Note that the volume of the female-to-male transsexual subject (FTM, in the male group, 51 years old) is in the male range, while the gender dysphoric male-to-female subject, who was not treated in any way (S7, in the MtF group, 84 years old), showed a male value for INAH3 volume. **b** Distribution of the INAH3 number of neurons among different groups. Bars represent means and standard errors of the mean (SEM). Statistically significant differences were found between men (M) and women (F) ($p < 0.029$) and between men (M) and male-to-female transsexual groups ($p < 0.002$). The FMT subject, in the male group, had a masculine INAH3 number of neurons and S7 subject, in the MtF group, had a similar number of neurons to the other transsexuals examined. From Garcia-Falgueras and Swaab [32] fig. 5 and 6, with permission.

In relation to sexual orientation, no difference was found in the size or number of neurons in the BSTc area, while for the INAH3 the volume has previously been found to be related to sexual orientation, being larger in heterosexual than in homosexual men [33]. In the MtF transsexuals group we found a completely female BSTc and INAH3. Until now we have only been able to obtain material from one female to male (FtM) transsexual, and his BSTc and INAH3 indeed turned out to have all the male characteristics. We were able to exclude the possibility that the reversal of sex differences in the BSTc and INAH3 were caused by changing hormone levels in adulthood, by including and comparing the results with a group of men that were gonadectomized because of

prostate carcinoma [32], and it therefore seems that we are dealing with a developmental effect. Our observations thus support the above-mentioned neurobiological theory about the origin of transsexuality. The size of the BSTc and the INAH3 and their number of neurons match the gender that transsexuals feel they belong to, and not the sex of their sexual organs, birth certificate or passport. Unfortunately, the sex difference in the BSTc volume does not become apparent until early adulthood [40], meaning that this nucleus cannot be used for the early diagnosis of transsexualism.

In transsexual MtF patients who receive hormonal treatment, some intermediate values, between those typical for men and women, have been found for lateralization and cognitive performance [41] and for the neuropeptide Y stained values in the INAH3 subdivision [32] (fig. 1), indicating a sex atypical development. The same was found with functional magnetic resonance imaging (fMRI) study in non-homosexual MtF transsexual people (i.e. erotically attracted to women), who were not treated hormonally: a number of brain areas in the transsexual hypothalamus were activated by pheromones in a sex-atypical way. Although the functional reactions in the hypothalamus to an estrogen-derived pheromone were predominantly female, MtF transsexual people also showed some characteristics of a male activation pattern [42].

Sexual Orientation

Sexual orientation in humans is also determined during early development, under the influence of our genetic background and factors that influence the interactions between the sex hormones and the developing brain [for references see 17]. The apparent impossibility of getting someone to change their sexual orientation [43] is a major argument against the importance of the social environment in the emergence of homosexuality, as well as against the idea that homosexuality is a lifestyle choice.

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

Abnormal hormone levels originating from the child itself during intrauterine development may influence sexual orientation, as is apparent from the large percentage of bisexual and homosexual girls with CAH. Between 1939 and 1960 some two million pregnant women in the US and Europe were prescribed diethylstilbestrol (DES) in order to prevent miscarriage. DES is an estrogen-like substance that actually turned out not to prevent miscarriage; furthermore, it also found, in small dosages, not only to give a slightly elevated risk of cervical cancer but also to increase the chance of bisexuality or homosexuality in girls.

The chance that a boy will be homosexual increases with the number of older brothers he has. This phenomenon is known as the fraternal birth order effect and is putatively explained by an immunological response by the mother to a product of the Y chromosome of her sons. The chance of such an immune response to male factors would increase with every pregnancy resulting in the birth of a son. Prenatal exposure to nicotine, amphetamine, or thyroid-gland hormones increases the chances of giving birth to lesbian daughters. A stressed pregnant woman has a greater chance of giving birth to a homosexual son. An interesting hypothesis is that the changes in androgen concentration during pregnancy as a result of environmental stress factors may influence the fetal central nervous system as an adaptive adjustment to the environment [44].

Although it has often been postulated that postnatal development is also important for the direction of sexual orientation, there is no solid proof for this. On the contrary, children who were born after artificial insemination with donor sperm and who were raised by a lesbian couple are heterosexually oriented [45]. There is also no proof for the idea that homosexuality is the result of a deficient upbringing, or that it is a 'lifestyle choice' or an effect of social learning [43]. It is curious, therefore, that some children are still forbidden to play with homosexual friends, an unthinkable attitude left over from the idea that homosexuality is 'contagious' or can be learned.

Sexual Orientation and the Brain

Several structural and functional differences in the brain have been described in relation to sexual orientation [for a review, see 17]. We found the first difference in the SCN, or brain clock, which turned out to be twice as large in homosexual compared with heterosexual men [46, 47].

In 1991, LeVay [47] reported that homosexual men, just like heterosexual women, have a smaller volume of the frontal part of the hypothalamus (INAH3). In 1992, Allen and Gorski reported that the anterior commissure of homosexual men is larger than that of heterosexual men. This structure, which is larger in women than in men, takes care of left-right connections within the temporal cortex, and is thus involved in sex differences in cognitive abilities and language. As shown by Savic and Lindström [48], this difference in size may possibly be related to the sex-atypical hemispheric

asymmetries observed in homosexual men and homosexual women [47, 48]. No differences were found in the BSTc volume or number of somatostatin neurons in homosexual compared to heterosexual men [38, 39].

Functional scanning has recently also shown differences in the hypothalamus in relation to sexual orientation: the hypothalamus of homosexual men turned out not to be as responsive to a classic antidepressant (fluoxetine) as that of heterosexual men, which suggests a different kind of activity of the serotonergic system [49].

There are some human studies that point to the presence of unconsciousness personal communication through pheromones. Savic and Lindström [48] used pheromone compounds derived from progesterone and excreted in perspiration in concentrations that are 10 times higher in men than in women and probed pheromones influence sexual behavior and stimulate activation in the hypothalamus of heterosexual women and homosexual men in the same way, but the one used in this study not elicit a PET response in the hypothalamus of heterosexual men. Apparently, heterosexual men are not stimulated by a male scent, which suggests that pheromones contribute to determining our behavior in relation to our sexual orientation [48]. In a follow-up study, lesbian women, as compared to heterosexual women, reacted in a sex-atypical, almost reciprocal way to pheromones. These observations, too, show that there are hypothalamic circuits that function in a way that depends on our sexual orientation.

Savic's previous studies raised the question of whether certain sexually dimorphic features in the brain, which are unlikely to be directly involved in reproduction, may differ between homosexual and heterosexual individuals. They showed hemispheric asymmetry, using volumetric MRI, and functional connectivity of the amygdala, using PET measurements of cerebral blood flow [47, 48]. Dichotic listening performance has also been found to show a greater right ear advantage in heterosexual men as compared to heterosexual women, while lesbian women were somewhat masculinized in their functional cerebral asymmetry [50].

These studies show sex-atypical cerebral asymmetry and functional connections in homosexual subjects that cannot be primarily linked to reproduction, and suggest a linkage between sexual orientation and neurobiological entities.

Conclusions

The human fetal brain develops in the male direction through a direct action of testosterone and in the female direction through the absence of such an action. During the intrauterine period, gender identity (the conviction of belonging to the male or female gender), sexual orientation, cognition, aggression and other behaviors are programmed in the brain in a sexually differentiated way. Sexual differentiation of the genitals takes place in the first 2 months of pregnancy, whereas sexual differentiation of the brain starts in the second half of pregnancy. This means that in the event of an

ambiguous sex at birth, the degree of masculinization of the genitals may not reflect the degree of masculinization of the brain.

Our observations on reversed sex differences in the brains of transsexual people support the idea that transsexuality is based on an opposite sexual differentiation of (1) sexual organs during the first couple of months of pregnancy, and (2) the brain in the second half of pregnancy. There is no proof that the social environment after birth has an effect on the development of gender or sexual orientation and hormonal changes during puberty do not seem to be responsible of the adult sexual identity and orientation, while the possible effects on sexual differentiation of the brain by endocrine disrupters in the environment and in medicines given to the pregnant mother should be investigated.

The differences observed in the INAH3 in relation to sexual orientation and gender identity and this structure's possible connection with the BSTc suggest that these two nuclei and the two earlier described nuclei that were found to be related to gender and sexual orientation, i.e. the SDN-POA (= intermediate nucleus = INAH1 and 2) and SCN, are all part of a complex network involved in various aspects of sexual behavior. Neurobiological research on sexual orientation and gender identity in humans is only just gathering momentum, but the evidence shows that humans have a vast array of brain differences. There is a need for further multidisciplinary research on the putative influence of testosterone in development, e.g. in individuals with complete androgen-insensitivity syndrome.

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