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SEXUAL DIFFERENTIATION OF THE HUMAN BRAIN

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SUMMARY

• *Sex differences in the structures of human hypothala-iiius and adjacent brain structures have been observed that seem to be related to gender, to gender problems such as transsexuality, and to sexual orientation, that is, heterosexu-al! ty and homosexuality. A Ithough these observations have yet to be confirmed, and their exact functional implications are far from clear, they open up a whole new field of structural-functional relationships in human brain research.* ***(BiomedRev*** *1997; 7:17-32)*

INTRODUCTION

• In analogy with observations in many mammalian species the human brain might well undergo sexual differentiation during its development due to an organizing effect of sex hormones, and such a structural organization might be the basis for functional sex differences (1 -3) and sex differences in neurological or psychiatric diseases (Table 1). In fact, remarkably little attention has been paid so far to the possible structural basis of the often pronounced sex differences in the epidemiology of such diseases (4). The proportions of cases range from more than 75% female in anorexia nervosa and bulimia to more than 75% male in dyslexia, sleep apnoea, Gilles de la Tourette's syndrome, and Kleine-Levin syndrome (Table 1). Not only might the number of cases of disorders show clear sex differences, but the signs and symptoms and the course of the disease might differ as well. Males not only suffer from schizophrenia 2.7 times more often than females, they are also prone to a more severe form of this disorder, experience an earlier onset, and exhibit more structural brain abnormalities. Relapses are more severe, and their response to neuroleptic medication is less favorable. On the other hand, sex-specific prevalence can vary with age, and females apparently have a greater susceptibility to acute food deprivation during the first trimester, as was evident from the children born in the Dutch hunger winter of 1944-1945 that resulted in 2.6 times more female than male schizophrenics. Another example is that following restricted posterior left-hemisphere lesions, 41% of the males and 11 % of the females developed aphasia, whereas manual apraxia was found in 6% of the females and 42% of the males. An important recent finding with respect to sexually dimorphic alterations in neurological diseases is the observation of Schultz *et al* (5). They showed a conspicuous neurofibrillary Alzheimer-like pathology in the infundibular nucleus and posterior median eminence in males over the age of 60, but not in females. The vessel-associated dystrophic neurites develop independently of Alzheimer changes in the neocortex. We propose to explain the lack of neurofibrillary changes in the mediobasal hypothalamus of females as an illustration of the way neurons that are activated are protected against the development of Alzheimer changes, a principle we

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Table 1. Ratios for females over males suffering from particular neurological and psychiatric diseases

Disease	% Female : Male
Anorexia nervosa	93 : 7
Bulimia	75 : 25
Schizophrenia following Dutch hunger winter	72 : 28
Anxiety disorder	67 : 33
Depression	63 : 37
Multiple sclerosis	58 : 42
Severe mental retardation	38 : 62
Autism	29 : 71
Stuttering	29 : 71
Schizophrenia	27 : 73
Dyslexia	23 : 77
Sleep apnoea	18 : 82
Gilles de la Tourette	10 : 90
Kleine-Levin*	<10 : 90

For references see (4).

** The few cases ofKleine-Levin syndrome witnessed in females are of doubtful validity (94).*

paraphrased as "use it or lose it". In the arcuate nucleus of postmenopausal women, luteinizing hormone-releasing hormone (LHRH) neurons are strongly activated (6).

THE HYPOTHALAMUSAND SEXUAL BEHAVIOR_____

• Sex differences in the hypothalamus and other limbic structures are thought to be the basis of sex differences in reproduction and sexual behavior, e.g. the menstrual cycle in women, gender identity (the feeling one is either male or female), gender identity problems (transsexuality) and sexual orientation (homosexuality and heterosexuality) (7-9). There is an extensive animal experimental literature showing that the hypothalamus is a key structure for male and female copulatory behavior (1, 10). Literature on hypothalamic structures involved in sexual orientation in experimental animals is, however, scarce and data on the hypothalamus in relation to gender identity in animals are, of course, non-existent. There are a lew studies in the medical literature implicating the hypothalamus and adjoining structures in various aspects of sexual behavior. Direct electrical or chemical stimulation of the septum may induce a sexually motivated state of varying degrees up to penile erection in men and building up to an orgasm in both sexes (11). Markedly increased sexual behavior was observed following the placement of the tip of a ventriculoperitoncal shunt into the septum in two cases (12). Meyers (13) described a loss of potency following lesion in the septo-fornico-hypothalamic region. Precocious puberty and hypersexuality have been reported following lesions in the posterior part of the hypothalamus, and hypogonadism is an early sign of pathology in the anterior part of the hypothalamus (14-16). In addition, there are a few case histories of changing sexual orientation, from heterosexual to pedophilic or homosexual, based on a lesion in the hypothalamus or in the temporal lobe, from which the amygdala has strong connections to the hypothalamus (17).

A German stereotactic psychosurgical study (18) reports on 22 male mainly pedo- or ephebophilic homosexuals (n=14) and 6 cases with disturbances of heterosexual behavior (hypersexuality, exhibitionism or pedophilia). In 12 homosexual patients, a lesion was made in the right ventromedial nucleus of the hypothalamus. In 8 patients, homosexual fantasies and impulses disappeared. In 6 patients, a "vivid desire for full heterosexual contacts" occurred after the operation. In one pedophilic patient bilateral destruction of the ventromedial nucleus was performed and he lost all interest in sexual activity after the operation. The heterosexual patients reported a significant reduction of their sexual drive. Although this report suggests that the human hypothalamus is involved in sexual orientation and sexual drive, the study is highly controversial, both from ethical and methodological points of view.

FACTORS INFLUENCING GENDER AND SEXUAL ORIENTATION

• Hardly any information is present yet about the factors that may influence gender and cause transsexuality in humans. Recently, Dessens (19) reported that some children born by women exposed to the anticonvulsants phenobarbital and diphantoin were found to be transsexuals. This happened remarkably often in view of the rarity of this disorder. This exciting observation on compounds known to alter steroid levels in animal experiments has to be examined further.

The determinants of human sexual orientation seem to be legion, as sexual orientation is influenced by genetic as well as non-genetic factors, as appeared from studies in families, twins and through molecular genetics (20-24). Hameref *al* (22) found linkage between DNA markers on the X chromosome and male sexual orientation. Linkage between the Xq28 markers and sexual orientation was detected for the gay male families, but not for the lesbian families (22, 24). Sex hormones during development also have an influence on sexual orientation judging by the increased proportion of bi- and homosexual girls that have adrenogenital syndrome (25, 26). Then there is diethylstilboestrol, a compound related to estrogens, that increases the occurrence of bi- and homosexuality in girls (27, 28). Maternal stress is thought to lead to increased occurrence of homosexuality in boys (29) and girls (30). As an interesting case his-

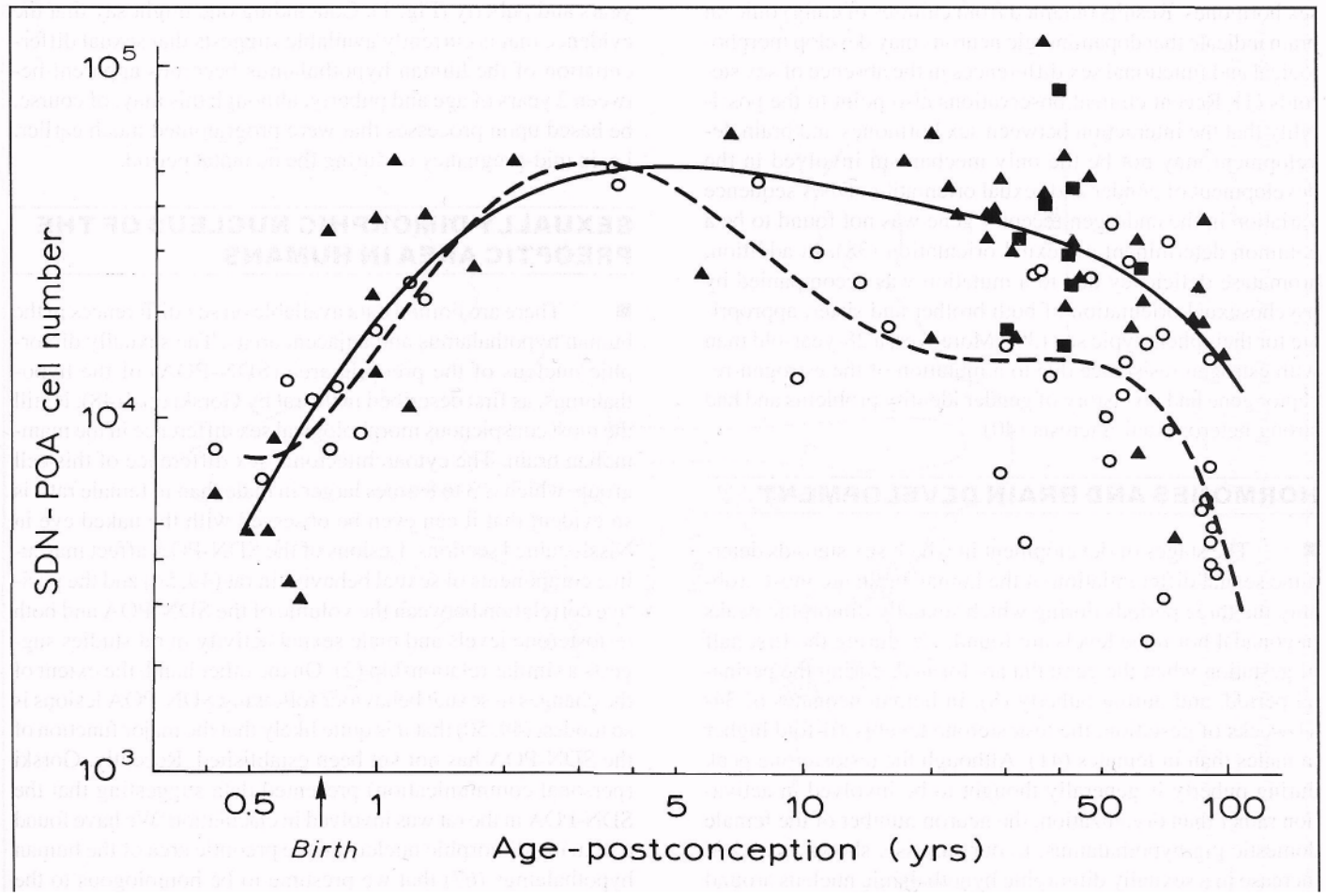


Figure 1. *Development and sexual differentiation of the human sexually dimorphic nucleus of the preoptic area (SDN-POA) of the hypothalamus. Log-log scale. Note that at birth SDN-POA is equally small in boys* (A) *and girls* (O) *and contains only about 20% of the cell number found at 2-4 years of age. Cell numbers reach a peak value around 2-4 years postnatally, after which a sexual differentiation occurs in the SDN-POA due to a decrease in cell number in the SDN-POA of women, whereas the cell number in men remains approximately unchanged up to the age of 50. The SDN-POA cell number in homosexual men* (•) *does not differ from that in the male reference group (for more data see Fig. 5). The curves are quintic polynomial functions fitted to the original data for males (drawn line) and females. From Ref. 46, with permission.*

tory of this factor, Weyl (31) had mentioned that Marcel Proust's mother was subjected to the overwhelming stress of the Paris commune during the fifth month of her pregnancy in 1871 and that Mary, Queen of Scots, the mother of the homosexual King of England, James I, toward the end of the fifth month of pregnancy had the terrifying experience that her secretary and special friend Riccio was killed. Although social factors are generally presumed to be involved in the development of sexual orientation (32), evidence in support of such an effect has not yet been reported. In fact, the observation that children raised by lesbian couples or by transsexuals generally have a heterosexual orientation (33-35) does not support the possibility of the social environment being an important factor for determining sexual orientation. Based on animal experiments it is expected that all compounds that influence neurotransmitter metabolism in development may affect sexual differentiation of the brain as well (1). Young adult male mice that were prenatally exposed to alcohol have a decreased preference for females and an increased preference for males as a partner (36). Exposure during development to some drugs, e.g. barbiturates, cause deviations in testosteron levels, persisting into adulthood. Exposure to other drugs, e.g. opiates, leads to behavioral changes despite apparently normal adult gonadal hormone levels (37).

In connection with this observation that points to an alternative mechanism of sexual differentiation, it is of great interest that there is recent animal experimental evidence for primary genetic control of sexual differentiation that does not involve

sex hormones. Results obtained from cultures of embryonic rat brain indicate that dopaminergic neurons may develop morphological and functional sex differences in the absence of sex steroids (1). Recent clinical observations also point to the possibility that the interaction between sex hormones and brain development may not be the only mechanism involved in the development of gender and sexual orientation. DNA sequence variation in the androgen receptor gene was not found to be a common determinant of sexual orientation (38). In addition, aromatase deficiency due to a mutation was accompanied by psychosexual orientation of both brother and sister, appropriate for their phenotypic sex (39). Moreover, a 28-year-old man with estrogen resistance due to a mutation of the estrogen-receptor gene had no history of gender identity problems and had strong heterosexual interests (40).

HORMONES AND BRAIN DEVELOPMENT

• The stages of development in which sex steroids determine sexual differentiation of the human brain are most probably the three periods during which sexually dimorphic peaks in gonadal hormone levels are found, *viz.* during the first half of gestation when the genitalia are formed, during the perinatal period, and during puberty (8). In human neonates of 34-41 weeks of gestation, the testosterone level is 10-fold higher in males than in females (41). Although the testosterone peak during puberty is generally thought to be involved in activation rather than organization, the neuron number of the female domestic pig hypothalamus, to our surprise, shows a twofold increase in a sexually dimorphic hypothalamic nucleus around puberty (42), which means that late organizational effects cannot be excluded. Few data arc available on the exact period in development when the human brain differentiates according to sex. Brain weight is sexually dimorphic from 2 years postnatally onwards, taking differences in body weight between boys and girls into account (43). The supposition of Dorner and Staudt (44) that structural sexual differentiation of the human hypothalamus would take place between 4 and 7 months of gestation was based only on the observation that the matrix layer around the third ventricle, in which the hypothalamic cells are presumed to have been formed, has disappeared by 7 months of gestation. Indeed, exhaustion of the matrix layer of the third ventricle begins in the 14-week-old embryo. A one-cell-layer ependyma appears from the 25- to the 28-week old embryo. Although the exhaustion of the matrix layer near the arcuate nucleus is present at 23 weeks of gestation, a multilayer ependyma remains here. No sex differences were observed in matrix exhaustion (45). Yet about 80% of the cells of the sexually dimorphic nucleus appeared to be formed postnatally (Fig. 1). In addition, it also becomes clear that cell death rather than cell division may be the most important mechanism in sexual differentiation of the nervous system (46,47). This mechanism takes place in the human sexually dimorphic nucleus between 4 years and puberty (Fig. 1). Concluding one might say that the evidence that is currently available suggests that sexual differentiation of the human hypothalamus becomes apparent between 2 years of age and puberty, although this may, of course, be based, upon processes that were programmed much earlier, i.e. in mid-pregnancy or during the neonatal period.

SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA IN HUMANS

• There are limited data available on sex differences in the human hypothalamus and adjacent areas. The sexually dimorphic nucleus of the preoptic area (SDN-POA) of the hypothalamus, as first described in the rat by Gorski *etal* (48), is still the most conspicuous morphological sex difference in the mammalian brain. The cytoarchitectonic sex difference of this cell group, which is 3 to 8 times larger in male than in female rats, is so evident that it can even be observed with the naked eye in Nissl-stained sections. Lesions of the SDN-POA affect masculine components of sexual behavior in rat (49, 50) and the positive correlation between the volume of the SDN-POA and both testosterone levels and male sexual activity in rat studies suggests a similar relationship (2). On the other hand, the extent of the changes in sexual behaviour following SDN-POA lesions is so modest (49, 50) that it is quite likely that the major function of the SDN-POA has not yet been established. Recently, Gorski (personal communication) presented data suggesting that the SDN-POA in the rat was involved in ejaculation. We have found a sexually dimorphic nucleus in the preoptic area of the human hypothalamus (67) that we presume to be homologous to the SDN-POA in the rat as judged from its sex difference in size and cell number, localization, cytoarchitecture and neurotransmitter/neuromodulatorcontent (Fig.2).

Immunocytochemical studies support such a homology between the SDN-POA in rat and human. Galanin- and galanin mRNA-containing neurons are present in the human SDN-POA (52, 53) (Fig.3) and in the same area in rat (54). In addition, the human SDN-POA contains thyrotropin-releasing hormone and cholecystokinin neurons (55; Zhou, unpublished results) similar to what has been reported in the rat (56). Moreover, the SON- 1 POA contains a high packing of preproenkephalin (57) and indeed, enkephalin is one of the markers that the rat and human SDN-POA have in common (van Leeuwen, personal communi- I cation). This nucleus is also present in rhesus monkey (58) but, to our knowledge, immunocytochemical studies of the SDN-POA peptide content have not yet been performed in these species.' 1

The human SDN-POA is identical to the intermediate nucleus (59). The first to describe the "intermediate nucleus" was Brockhaus (60). However, this term has become confusing, since Feremutsch (61) described a different "intermediate nucleus". He used this term for the accessory nuclei, i.e. the

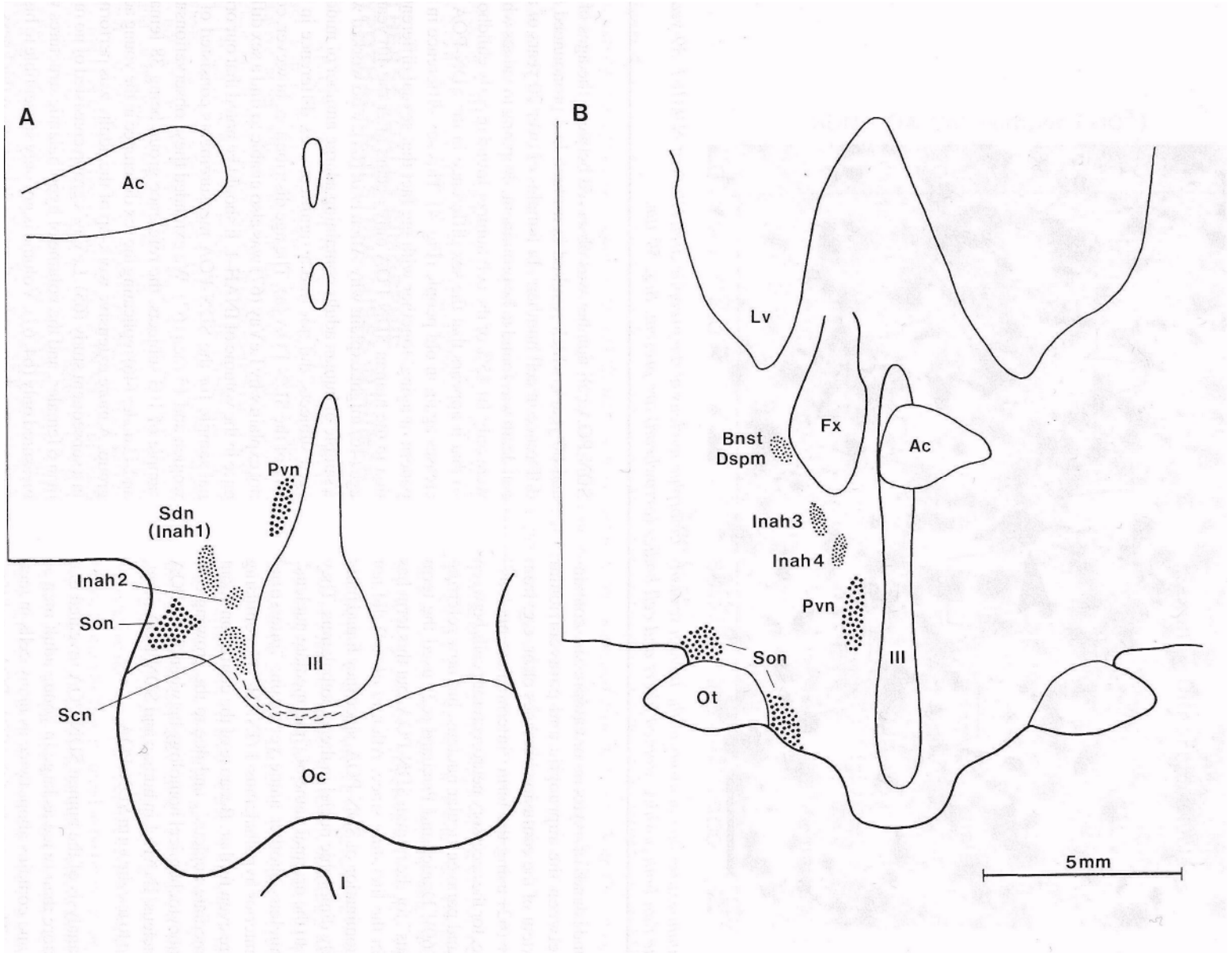


Figure 2. *Topography of the sexually dimorphic structures in the human hypothalamus. A is more rostral than B. Ill, third ventricle; Ac, anterior commissure; I, inifundibulum; Lv, lateral ventricle; Oc, optic chiasm; Sen, suprachiasmatic nucleus; Sdn, sexually dimorphic nucleus of the preoptic area; Pvn, paraventricular nucleus; Son, supraoptic nucleus; Bnst/Dspm, darkly staining posteriomedial component of the bed nucleus of the stria terminalis; Inahl-4. interstitial nucleus of the anterior hypothalamus 1-4*

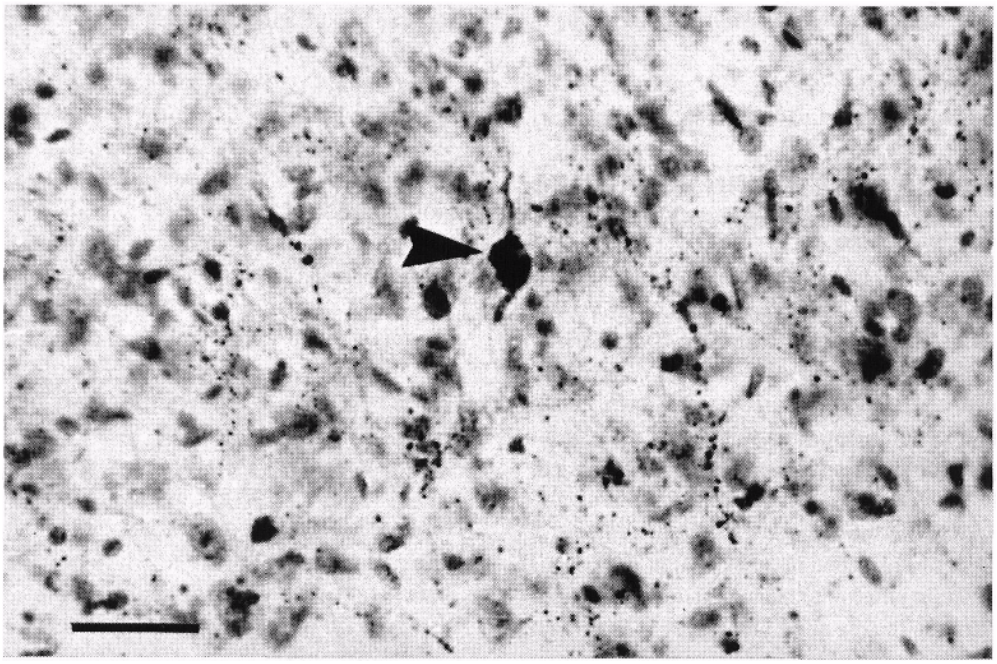


Figure 3. Galanin-expressing neurons in the human sexuall\ dimorphic nucleus of the preoptic area (Patient N 93161, 39-year-old male). Note that both positive stained fibers and cell bodies (arrowhead) are present. Bar, 50 jjm.

scattered cells and islands of oxytocin- or vasopressin- containing neurons between the supraoptic and paraventricular nucleus. The extent of the confusion becomes clear, e.g. from Morion's paper (62) using the term "intermediate nucleus" again by mistake, for the accessory neurosecretory cells between the supraoptic and paraventricular nucleus, but now referring to Brockhaus (60)! Daniel and Prichard (63) used the term "preoptic nucleus" for the human SDN-POA, but this term has not been used in the literature since. Alien *et al* (64) did not conform the denomination of SDN-POA, since they found more than one sexually dimorphic nuclei in the hypothalamus. They did not go back to the original name of"'intermediate nucleus" but gave this nucleus another name yet again: "interstitial nucleus of the anterior hypothalamus-1 (INAH-1)", confusing the nomenclature even further. Because of the confusion about the term "intermediate nucleus", and due to the growing evidence of immunocytochemical homology between SDN-POA /intermediate nucleus/INAH-1 in human and SDN-POA in rat, we will continue to use the term SDN-POA.

Morphometric analysis of the human SDN-POA revealed that the volume is more than twice as large in young adult men as it is in women, and contains about twice as many cells in men (46, 66) (Fig. 4). The magnitude of the SDN-POA sex difference was found not to remain constant throughout adulthood, but to depend on age (Fig. 4). In males, a major reduction in SDN-POA cell number was observed between the ages of 50 and 60 years, which resulted in a much less pronounced sex difference in cell numbers. In females of over 70 years of age cell death was found to be prominent, dropping to values which were only 10-15% of the cell number found in early childhood, so that it appears that the sex difference in the SDN-POA increases again in old people (Fig. 4). This sex difference in the pattern of aging, together with the fact that sexual differentiation in the human SDN-POA only occurs after the 4th year of age (46) might explain why Alien *et al* (64), who worked with a sample of human adults containing a large number of middle-aged subjects, did not find a significant sex difference in the size of the SDN-POA (8). The age distribution, however, coud not explain why LeVay (67) was also unable to find a sex difference in the volume of INAH-1. It should be noted that our original sample for the SDN-POA measurements consisted of 18 women and 13 men (67). We extended these observations to a sample of 103 subjects, the reference group being 38 females and 42 males (46) replicating the sex difference in the young adult group. A more extensive work-up of the adults was performed in a subsequent study (65). LeVay's group consisted of no more than 6 females, and the volume of hypothalamic structures was measured only (64, 67). Volume is not only susceptible to histological procedures and methods such as section thickness, but also to various pre- and postmortem factors such as differences in agonal state and fixation time (68). It is therefore essential to

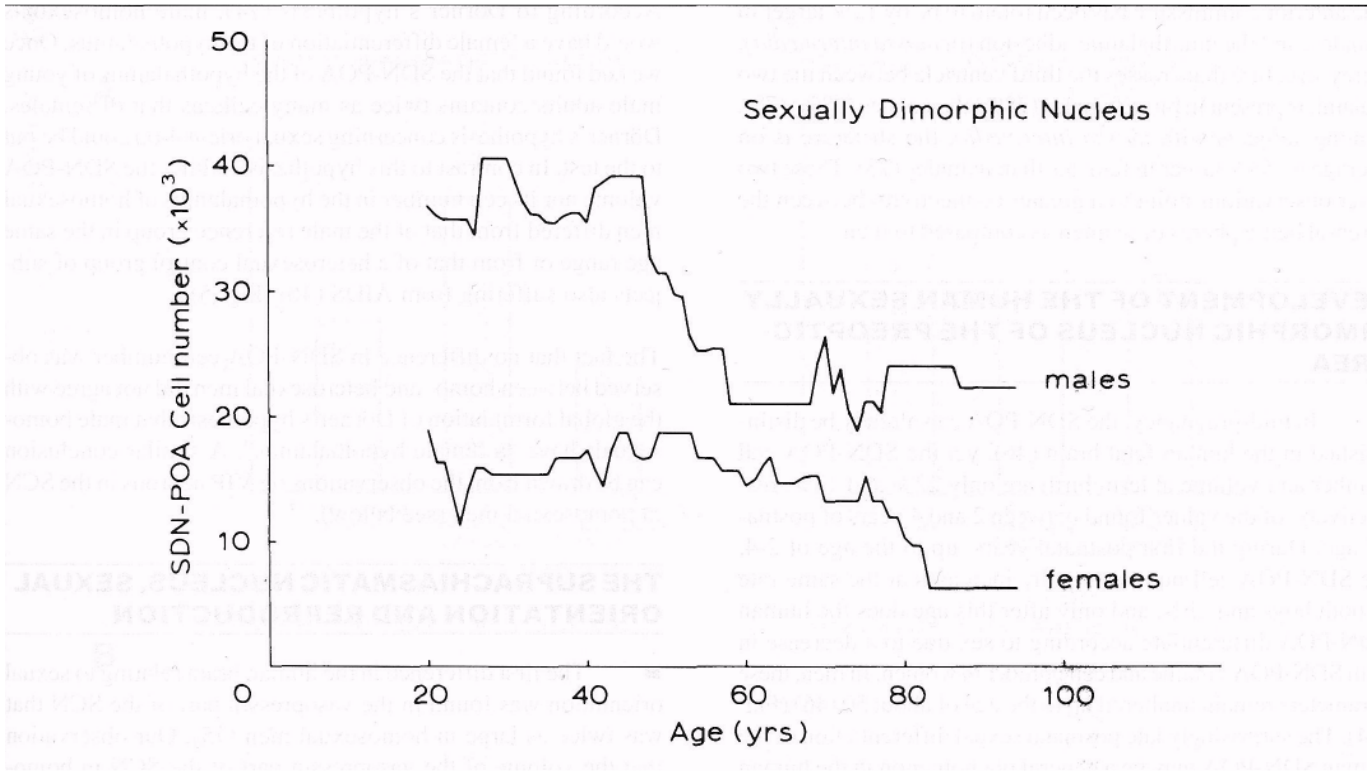


***Figure** 4. Age-related changes in the total cell number of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves. Note that in males SDN-POA cell number steeply declines between the age of 50-60 years, whereas in females, from the age of about 50 years, a more gradual cell loss is observed, which continues up to old age. These growth curves demonstrate that the reduction in cell number in the human SDN-POA in senescence is a non-linear, sex-dependent process. From Ref. 65, with permission.*

include data on total cell numbers ofhypothalamic nuclei, since this parameter is not influenced by such factors.

OTHER SEXUALLY DIMORPHIC HYPOTHALAMIC STRUCTURES IN HUMAN

• Alien *et* a/(64) described two other cell groups, INAH-2 and -3, in the preoptic-anterior hypothalamic area of humans that were larger in the male brain than in the female brain. It is unclear which nuclei in the rat are homologous to the INAH-2 and -3 because nothing is known about their neurotransmitter content so far. Since no immunocytochemistry was performed it is not clear whether the nuclei should be considered either islands of the paraventricular nucleus (PVN) or bed nucleus of *stria terminally* (BNST). or separate anatomical entities. LeVay (67) could not confirm the sex difference in INAH-2, but found such a difference in INAH-3. The discrepancy between LeVay's (67) data and those of Alien *et al* (64) in INAH-2 size can be Cully explained by an age-related sex difference in this nucleus. INAH-2 only shows a sex difference after the child-bearing age and in a 44-year-old woman who had had a hysterectomy involving the removal of ovaries 3 years prior to her death (64). This seems to be an example of a sex difference depending on circulating levels of sex hormones, i.e. a difference based on a lack of *activating* effects of sex hormones in menopause and not on the *organizing* effects of sex hormones in development.

Another clear sex difference was described by Alien and Gorski (69) in what they called the darkly staining posteromedial component (dspm) of the BNST. The volume of the BNST-dspm was found to be 2.5 times larger in males than in females.

The vasopressin-containing part of the suprachiasmatic nucleus (SCN) showed a sex difference only in shape, but not in volume or vasopressin cell number The shape of the SCN was elongated in women and more spherical in men (51). However, the vasoactive intestinal polypeptide (VlP)-containing subnucleus of the human SCN was found to be twice as large in young men (10-30 years) as in young women, and contained twice as many cells (70). From the age of 40 onwards this sex difference was reversed (71). These observations show again how important the factor age is in the case of sexual dimorphism of the human brain. It should be noted that sex differences in circadian time keeping have been described (72).

The anterior commissure has been found to be by 12% larger in females, and the interthalamic adhesion *(ormassa intermedia),* a grey structure that crosses the third ventricle between the two thalami, is present in more females (78%) than males (68%) (73). Among subjects with *massa intermedia,* the structure is on average by 53% larger in females than in males (73). These two latter observations point to a greater connectivity between the cerebral hemispheres of women as compared to men.

DEVELOPMENT OF THE HUMAN SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA

• In mid-pregnancy, the SDN-POA can already be distinguished in the human fetal brain (46), yet the SDN-POA cell number and volume at term birth are only 22% and 18%, respectively, of the values found between 2 and 4 years of postnatal age. During the first postnatal years, up to the age of 2-4, the SDN-POA cell number rapidly increases at the same rate in both boys and girls, and only after this age does the human SDN-POA differentiate according to sex due to a decrease in both SDN-POA volume and cell number in women. In men, these parameters remain unaltered up to the age of about 50 (46) (Fig. 1,4). The surprisingly late postnatal sexual differentiation of the human SDN-POA may be a general phenomenon in the human brain, as it seems as if the sex difference in the volume of the BNST-dspm does not occur until adulthood (69). Together these data support the notion that sexual differentiation of the human hypothalamus takes place after the perinatal period and before adulthood rather than during mid-gestation, although it is possible that the pre- or perinatal testosterone peak programmes cell death a few years later.

THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA AND SEXUAL ORIENTATION

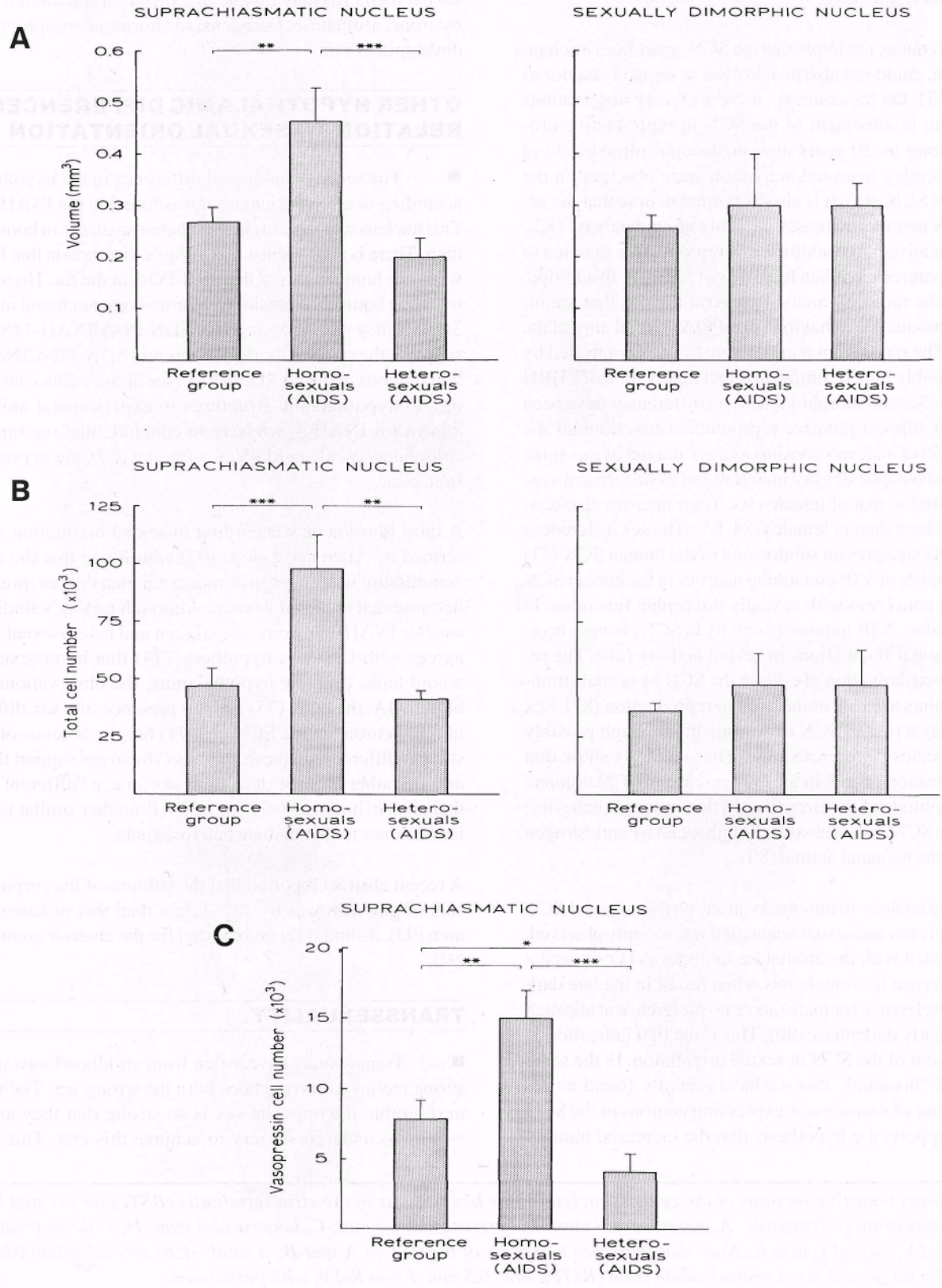
• A prominent theory about the development of heterosexual/homosexual orientation is that it develops as a result of an interaction between the developing brain and sex hormones. According to Dorner's hypothesis (74), male homosexuals would have a female differentiation of the hypothalamus. Once we had found that the SDN-POA of the hypothalamus of young male adults contains twice as many cells as that of females, Dorner's hypothesis concerning sexual orientation could be put to the test. In contrast to this hypothesis, neither the SDN-POA volume nor its cell number in the hypothalamus of homosexual men differed from that of the male reference group in the same age range or from that of a heterosexual control group of subjects also suffering from AIDS (46) (Fig. 5).

The fact that no difference in SDN-POA cell number was observed between homo- and heterosexual men did not agree with the global formulation of Dorner's hypothesis that male homosexuals have "a female hypothalamus". A similar conclusion can be drawn from the observations on VIP neurons in the SCN in homosexual men (see below).

THE SUPRACHIASMATIC NUCLEUS, SEXUAL ORIENTATION AND REPRODUCTION

• The first difference in the human brain relating to sexual orientation was found in the vasopressin part of the SCN that was twice as large in homosexual men (75). Our observation that the volume of the vasopressin part of the SCN in homosexual men was 1.7 times as large and contained 2.1 times as many cells as the SCN of the male reference group (Fig. 5) (75) also implied that the difference in SCN volume could not be attributed to differences in shrinkage of hypothalamic tissue during the histological procedure. The difference in the vasopressin cells of the SCN in relation to sexual orientation seems to be rather specific, since the number of VIP neurons in the SCN of homo- and heterosexual men was not different (76). The SCN is, indeed, the clock of the human brain, and regulates circadian and circannual changes (77, 78). Differences in the SCN between homosexual and heterosexual men may thus go together with differences in circadian rhythms. Recently it was found that gay men arise and retire earlier than heterosexual men (79). For circadian endocrine differences between homosexual

*Figure 5. A. Volume of the human suprachiasmatic nucleus (SCN) and sexually dimorphic nucleus of the preoptic area (SDN- -^ POA) as measured in 3 groups of adult subjects: (1) a male reference group (n=18); (2) male homosexuals who died of AIDS (n=10) and (3) heterosexuals who died of AIDS (n=6; 4 males and 2 females). The values indicate medians and the standard deviation of the median. The differences in the volume of the SCN between homosexuals and the subjects from both other groups are statistically significant. (Kruskal-Wallis multiple comparison test, *P < 0.05;* **P < 0.07; ***P < *0.001). Note that none of the parameters measured in the SDN-POA (A,* ***B)*** *showed significant differences among the 3 groups (P always > 0.4). B. Total number of cells in the human SCN and SDN-POA. The SCN in homosexual men contains 2.1 times as many cells as in the reference group of male subjects and 2.4 times as many cells as the SCN in heterosexual AIDS patients. C. The number of vasopressin (VP) neurons in the human SCN. The human SDN does not contain VP-producing cells. The SCN in homosexual men contains, on average, 1.9 times as many VP-producing neurons as the reference group of male subjects and 3.6 times as many VP neurons as the SCN in heterosexual AIDS patients. Note that the SCN of heterosexual individuals who died of AIDS contains less VP cells than the subjects from the reference group. From Ref. 75, with permission.*


A
SUPRACHIASMATIC NUCLEUS
SEXUALLY DIMORPHIC NUCLEUS
Volume (mm³)
0.6
0.5
0.4
0.3
0.2
0.1
**
***
Reference group
Homo-sexuals (AIDS)
Hetero-sexuals (AIDS)
B
SUPRACHIASMATIC NUCLEUS
SEXUALLY DIMORPHIC NUCLEUS
Total cell number (x10³)
125
100
75
50
25
***
**
Reference group
Homo-sexuals (AIDS)
Hetero-sexuals (AIDS)
C
SUPRACHIASMATIC NUCLEUS
Vasopressin cell number (x10³)
20
15
10
5
*
**
***
Reference group
Homo-sexuals (AIDS)
Hetero-sexuals (AIDS)

and heterosexual controls see also (80), although not interpreted by the authors in this way.

This does, of course, not imply that the SCN, apart from its circadian function, could not also be involved in sexual behavior as suggested (67). On the contrary, there are many observations suggesting an involvement of the SCN in reproductive processes. As long as 20 years ago, post-coital ultrastructural changes indicating neuronal activation were observed in the female rabbit SCN (81). It is also important to note that the activity of SCN neurons increases suddenly around puberty (82), which is indicative of the addition of a reproductive function to the already matured circadian functions of rat SCN. In addition, el'fercnts of the rat SCN innervate several regions that are involved in reproductive behaviors, e.g. POA, medial amygdala, and BNST. The rat ovarian reproductive cycle is controlled by the SCN, possibly by VIP fibres *via* direct.innervation of LHRH neurons (83). Several morphological sex differences have been reported that support putative reproductive functions of the SCN. The SCN of male rats contains a larger amount of axo-spine synapses, postsynaptic density material, and asymmetrical synapses compared to that of female rats. Their neurons also contain more nucleoli than in females (84, 85). The sex differences in shape of the vasopressin subdivision of the human SCN (51) and in the number of VIP-con taming neurons in the human SCN (70) are also consistent with sexually dimorphic functions. In seasonal breeders, VIP-immunoreactivity in SCN changes in relation to seasonal fluctuations in sexual activity (86). The recently observed activation *ofc-fos* in the SCN by sexual stimulation also points to a role of the SCN in reproduction (87). Sex hormones play a role in SCN development, although possibly subject to species differences (88). These authors show that neonatal castration results in a 62% decrease in SCN volume. Also, the amplitude of the circadian rhythm in sexual behavior, for which the SCN is the substrate, is enhanced by antiestrogen treatment of the neonatal animal (89).

An interesting analogy to our observations on the enlarged SCN in homosexual men and sexual-orientation was recently observed. Male rats treated with the aromatase inhibitor ATD showed a partner preference for female rats when tested in the late dark phase and a preference for male rats or no preference at all when tested in the early dark phase (90). This is the first indication of the involvement of the SCN in sexual orientation. In the same ATD treated "bisexual" rats we have recently found an increased number of vasopressin-cxpressing neurons in the SCN (91). This supports the hypothesis that the increased number of vasopressin neurons that we observed in the SCN of homosexual men (75) may be due to a different interaction of testosterone, aromatase, estrogens, sex hormone receptors and the developing brain.

OTHERHYPOTHALAMIC DIFFERENCES IN RELATION TO SEXUAL ORIENTATION

• The second anatomical difference in the hypothalamus according to sexual orientation was found in the INAH-3 (67). This nucleus was twice as large in heterosexual as in homosexual men. There is no evidence for LeVay's assumption that INAH-3 would be homologous to the SDN-POA in the rat. Thyrotropin-releasing hormone-containing neurons were not found in INAH-3, while they were present in the SDN-POA/INAH-1 (55). This supports the possibility that the human SDN-POA/INAH-1 is homologous to the rat SDN-POA (see above). Since no homology to hypothalamic structures in experimental animals is known for INAH-3, we have to conclude that the functional consequences, also of LeVay's finding (67), are at present far from clear.

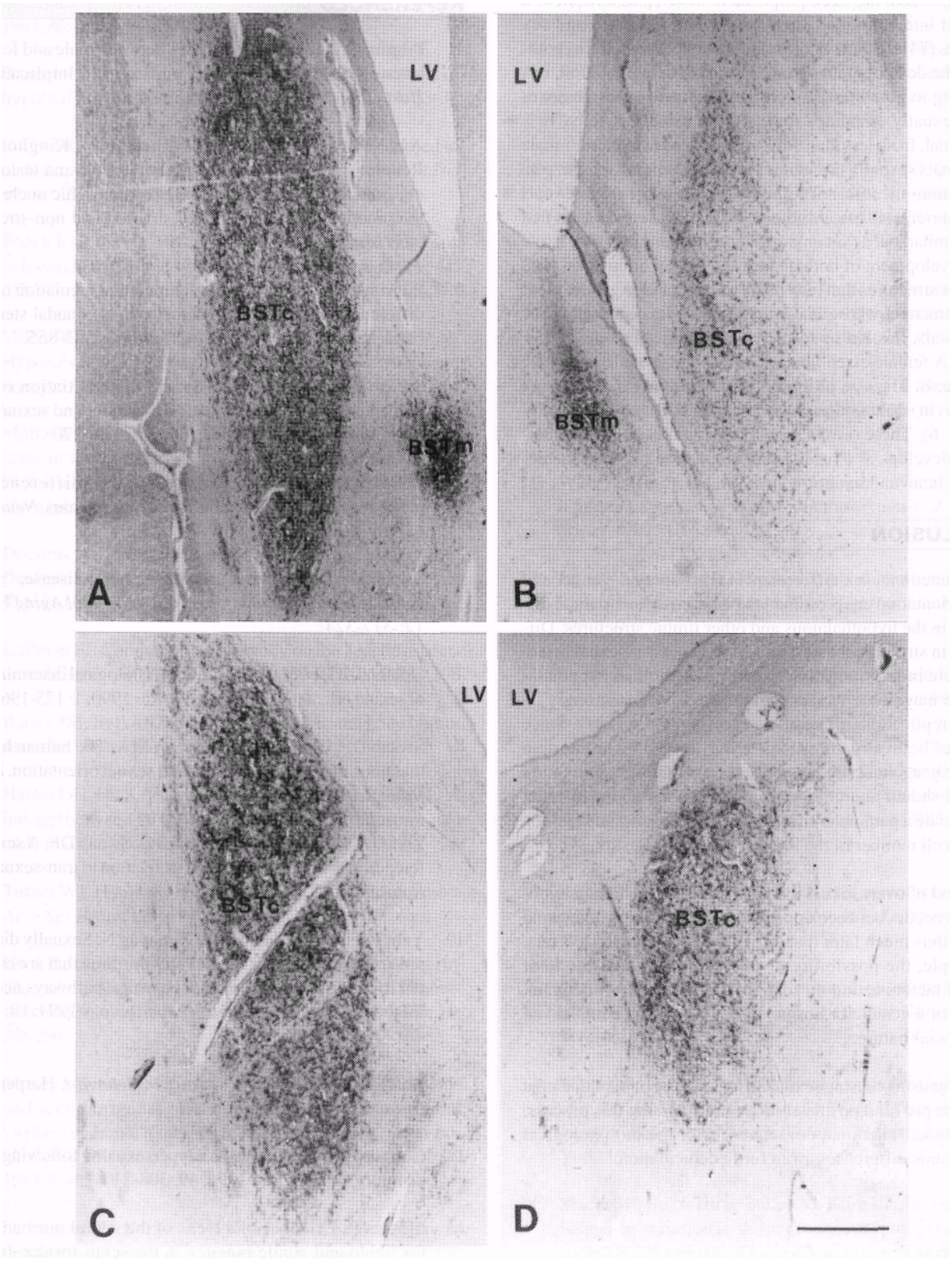
A third idiosyncrasy according to sexual orientation was described by Alien and Gorski (92) who found that the anterior commissure was larger in homosexual men than in (presumed) heterosexual men and women. Although LeVay's finding of a smaller INAH-3 in homosexual men and heterosexual women agrees with Dorner's hypothesis (74) that homosexual men would have a female hypothalamus, the observations in the SDN-POA, the SCN (75) and the presence of a sex difference in VIP neurons in the SCN (70, 71) but the absence of a gay-straight difference in these neurons (76) do not support this idea and are rather in favor of a "third sex", i.e. a "different" hypothalamus in homosexual men which is neither similar to that in females, nor to that in male heterosexuals.

A recent abstract reported that the isthmus of the corpus callosum of gay men was by 13% larger than that of heterosexual men (93), a similar result reported for the anterior commissure (92).

TRANSSEXUALITY

• Transsexuals have, often from childhood onwards, the strong feeling of having been born the wrong sex. Their desire to resemble the opposite sex is so strong that they are even willing to undergo surgery to achieve this end. This gender

Figure 6. *Representative sections of the central nucleus of the bed nucleus of the stria terminalis (BSTc) innervated by vasoqctive intestinal polvpeptide. A, heterosexual man; B, heterosexual woman; C, homosexual man; D, male-to-female transsexual; LV, lateral ventricle. Note that there are two parts of the BST in A and B: a small-sized medial subdivision (BSTni) and a large ova I-sized central subdivision (BSTc). Bar, 0.5 mm. From Ref 9, with permission.*


LV
BSTc
BSTm
A
LV
BSTc
BSTm
B
LV
BSTc
C
LV
BSTc
D

identity problem has been proposed to develop as a result of a disturbed interaction between the developing brain and sex hormones (74). In view of the relationship between the hypotheses on the development of gender and sexual orientation, it is interesting to note that 60% of the male-to-female transsexuals are sexually orientated towards males and that some 10% are bisexual. In no less than 95% of the cases are female-to-male transsexuals sexually orientated towards women (Gooren, personal communication). The high proportion of transsexuals sexually orientated towards their own genetic sex indicates that indeed similar, but as yet unknown, mechanisms may play arole in the development of both gender and sexual orientation. The search for structures that may be directly related to gender identity, i.e. structures whose anatomy is "female" in genetically male transsexuals, has led us to the central nucleus of the BNST (BSTc). A female-sized nucleus was found in male-to-female transsexuals. The size of the BSTc was not influenced by sex hormones in adulthood and was independent of sexual orientation (Fig. 6). These results support the hypothesis that gender-identity develops as a result of an interaction between the developing brain and sex hormones (9).

CONCLUSION

• Functional sex differences in reproduction, gender and sexual orientation are presumed to be based on anatomical differences in the hypothalamus and other limbic structures. Differences in structure that are related to gender or sexual orientation were indeed recently reported in the human hypothalamus. The magnitude of such differences depends strongly on age, and replication of these data is certainly necessary. Since the size of brain structures may be influenced by premortem factors, e.g. agonal state, and postmortem factors, e.g. fixation time, one should not only perform volume measurements, but also estimate a parameter that is not dependent on such factors, i.e. total cell number of the brain structure in question.

The period of overt sexual differentiation of the human hypothalamus occurs between approximately four years of age and puberty, thus much later than is generally presumed. It offers, in principle, the possibility of interaction of a multitude of postnatal factors acting on sexual differentiation of the brain, not only of a genetic or hormonal, but also of a chemical and psychosocial nature.

The mechanisms causing sexual differentiation of hypothalamic nuclei, the pre- and postnatal factors influencing this process, and the exact functional consequences of the morphological hypothalamic differences await further elucidation.

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