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Gender and Sexual Orientation in Relation to Hypothalamic Structures

Key Words
Hypothalamus
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Abstract
Animal experiments have provided evidence for the presence of sex differences from the synaptic level up to behaviour. Although sex differences in the human brain may have been presumed implicitly since the days of Aristotle, research on the presence of functional and structural sex differences of the human brain started only relatively recently. The most conspicuous sex difference in the mammalian brain was described by Gorski et al. [1978] in the preoptic area (POA) of the rat hypothalamus. We found that the volume of a putative homologue of this sexually dimorphic nucleus (SDN) in the adult human hypothalamus was more than twice as large in men as in women and contained about twice as many cells. Recently a similar sex difference and volume has been described for the human bed nucleus of the stria terminalis and 'interstitial nuclei of the hypothalamus' (INAH). Sexual differentiation of the hypothalamus was generally believed to take place between 4 and 7 months of gestation. A life span study on the SDN of more than 100 subjects revealed, however, that only after the age of 2–4 years postnatally sexual differentiation becomes manifest by a decrease in volume and cell number in the female SDN. If sexual differentiation of the brain indeed takes place postnatally, not only chemical and hormonal factors may influence this process but also social factors.

A prominent theory on the development of sexual orientation is that it develops as a result of an interaction between the developing brain and sex hormones. According to Dörner's hypothesis, male homosexuals have a female differentiation of the hypothalamus. This hypothesis was not supported by our observations on the SDN. Neither the SDN volume nor the cell number in the hypothalamus of homosexual men differed from that of heterosexual men. However, a difference in SCN cell number was observed in relation to sexual orientation. The volume and cell number of the SCN of homosexual men was twice as large as that of a reference group. 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 the cell numbers subsequently decline to the adult value, which is about 35% of the peak value. The observation that an SCN similarly enlarged as that observed in homosexual men was present in a woman with Prader-Willi syndrome, a congenital LHRH deficiency in which sex hormones are very low, suggests that at some stage of brain development the interaction with sex hormones might be essential for the programmed SCN cell death.

In conclusion, marked sex differences in the human hypothalamus are found in the SDN and BST, and an enlarged SCN is present in homosexual men. The functional implications of such differences in relation to gender and sexual orientation are, however, far from clear at the moment.

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Introduction

Between 4 years of age and puberty we become aware of our gender identity (i.e. being a boy or a girl) and our sexual orientation (i.e. being heterosexual or homosexual). All possible combinations of gender identity and forms of sexual orientation have been described. Some combinations are less valued than others by society, and because of this some have tried to change gender identity or sexual orientation; though with little success. This is probably because gender identity and sexual orientation are fixed in the brain. The present paper gives some recent evidence for this concept.

Sexual Differentiation of the Human Hypothalamus

Following observations in many mammalian species, one is inclined to believe that the human brain undergoes sexual differentiation during its development, due to an organizing effect of sex hormones. However, the stage of development at which sex steroids determine sexual differentiation of the human brain is unknown, as are the exact functional implications of such hormonal actions in relation to gender and sexual orientation. There are three sex-dimorphic peaks in gonadal hormone levels which could be of importance for these processes: during the first half of gestation, when the genitalia are formed [1]; in the perinatal period; and during puberty [2]. Also, total brain weight in adults is sexually dimorphic [3]. The brain area thought to be the primary substrate of sex differences in reproduction, gender identity and sexual orientation is the hypothalamus [4–6]. The supposition that structural sexual differentiation of the human hypothalamus takes place between 4 and 7 months of gestation [7] was based on the observations that: (1) during this period various hypothalamic cell groups (the supraoptic, ventromedial and paraventricular nucleus) can be distinguished histologically (although sex differences in these nuclei were not reported); and (2) the matrix layer surrounding the third ventricle, in which the cells are formed, has disappeared by 7 months of gestation. Later it became clear that cell death rather than cell division may be the most important mechanism in the sexual differentiation of the nervous system [8, 9].

Sexually Dimorphic Nucleus

The sexually dimorphic nucleus (SDN) of the preoptic area of the hypothalamus, as first described in the rat by Gorski et al. [10], is the most conspicuous morphological sex difference in the mammalian brain. The cell group, which is 3–8 times larger in male rats than in female rats (fig. 1), has such a clear cytoarchitectonic sex difference that it can be seen with the naked eye in Nissl-stained sections. Lesions of the SDN affect masculine components of sexual behaviour in the rat, and SDN size is correlated to male sexual activity [11–13]. On the other hand, the changes in sexual behaviour following SDN lesions are so modest that the major function of the SDN has probably not yet been revealed.

Human SDN

We have found an SDN in the preoptic area of the human hypothalamus that is (judging by its localization and cytoarchitecture) probably homologous to that in the rat [14], although proof for such homology, on the basis of transmitter content, afferents and efferents is lacking (fig. 2). Morphometric analysis of the human SDN revealed that the volume is more than twice as large in adult men as in women, and contains approximately twice as many cells in men (fig. 3). The human SDN corresponds to the intermediate nucleus described by Braak and Braak [16] and to the intermediate lateral hypothalamic area described by Brockhaus [17] and Feremutsch [18]. However, it does not correspond with the intermediate nucleus of an earlier paper of Feremutsch [19], who described the islands of neurosecretory cells between the supraoptic and paraventricular nucleus (SON, PVN) by this name. Because of this confusion, we will use the name SDN in the present paper.

The sex difference which we observed in the SDN was not present in other hypothalamic nuclei (fig. 1). The magnitude of the sex difference did not remain constant throughout adulthood, but depended on age (fig. 3). In males, a major reduction in SDN cell number was observed between the age of 50 and 60 years, so that the sex difference became smaller. In females over 70 years of age, cell death was found to be more prominent than in males, dropping to values which were only 10–15% of the cell number found in early childhood. Thus, the sex difference in SDN increases again in old people [15]. This sex difference in the pattern of ageing, and the fact that sexual differentiation in the human SDN only occurs after the fourth year of age [9] might explain why Allen et al. [20], who had a sample of human adults biased for age, did not find a significant sex difference in the size of the SDN (which they called interstitial nucleus of the anterior hypothalamus 1 (INAH-1). In that study [20], 40% of the adult subjects came from the age group in which the SDN sex difference is minimal, compared with 29% in our study [15]. Moreover, the age group of elderly subjects (over 70 years of age) was under-repre-

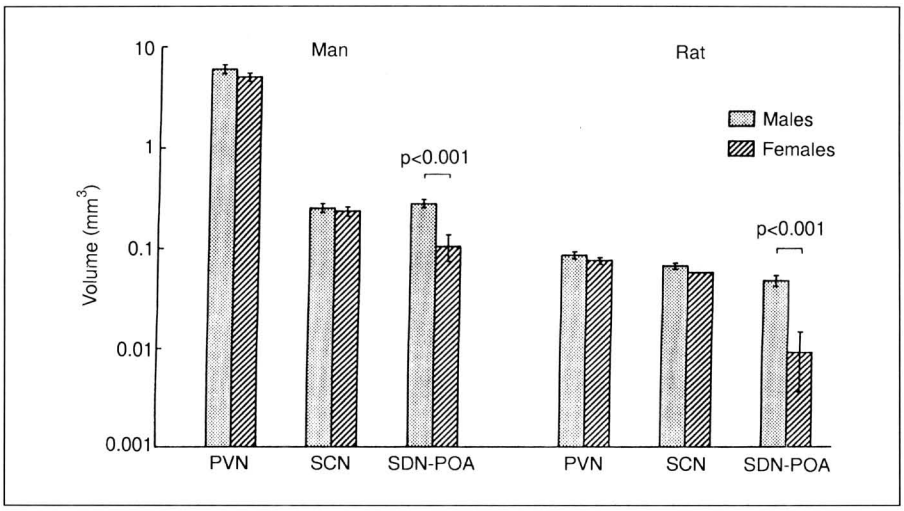


Fig. 1. Volumes of hypothalamic regions in young adult rats (2–5.5 months) and in man (20–40 years). Values represent the mean volume (± SEM) from one hemisphere. The variance of the suprachiasmatic nucleus (SCN) volume in female rats could not be determined. The sexual differences in the volume of the sexually dimorphic nucleus in the preoptic area of the hypothalamus (SDN-POA) were statistically significant, both in man and in the rat. (From Hofman and Swaab [15] with permission.)

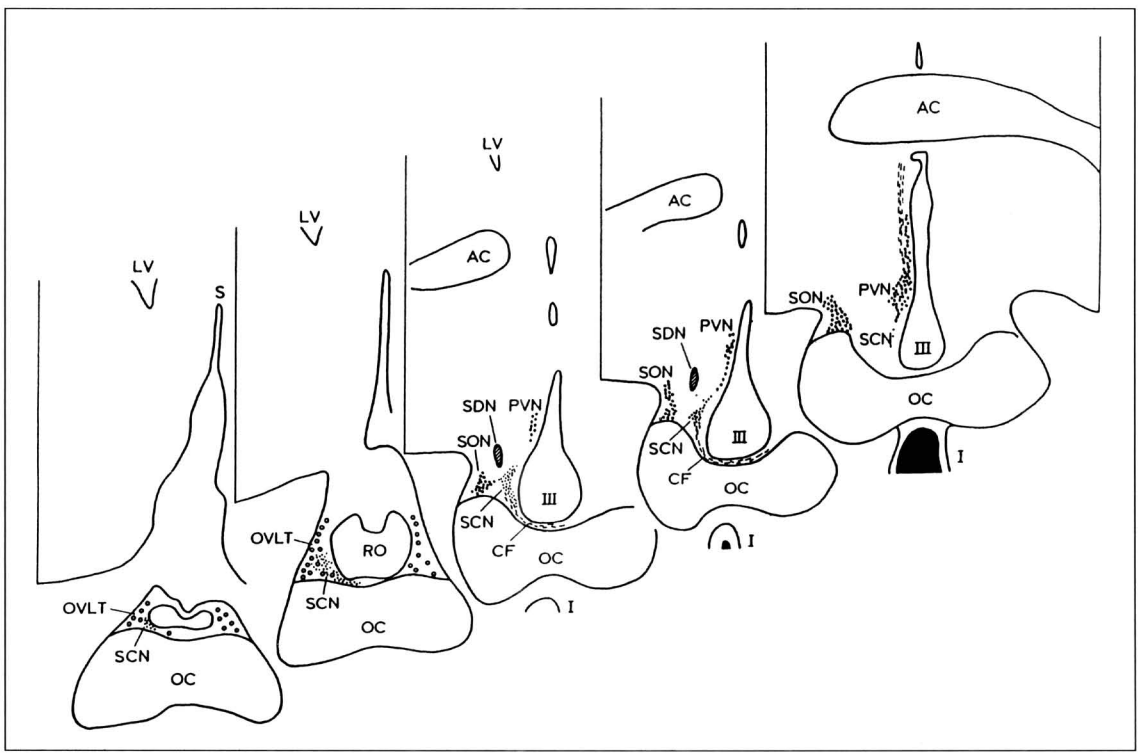


Fig. 2. Topography of the sexually dimorphic nucleus (SDN) in the preoptic area of the human hypothalamus. Third ventricle, III; anterior commissure, AC; infundibulum, I; lateral ventricle, LV; optic chiasm, OC; organum vasculosum of the lamina terminalis, OVLT; recessus opticus, RO; septum, S; suprachiasmatic nucleus, SCN; paraventricular nucleus, PVN; supraoptic nucleus, SON; commissural fibres of the suprachiasmatic nucleus, CF. (From Swaab and Fliers [14] with permission; copyright 1985 by the AAAS.)

sented in Allen's study [20]: 20% compared with 37.5% in the case of a proportional distribution of all ages. In our study, 32% of the subjects belonged to this old age group. It seems thus likely that Allen et al. [20] were unable to establish a sex difference in the INAH-1 (i.e. SDN) because they used a biased sample. A further argument for this assumption is that, if we [15] had studied only subjects of the age distribution of Allen's study, the sex difference in SDN volume would have been reduced from 2 to only 1.4 times, and this difference would not have been statistically significant.

Moreover, the sex difference in the SDN emerges only between the ages of 4 years and puberty [9]; therefore the brains of the 5-year-old boy and 4-year-old girl (she indeed had by far the largest volume of the entire series of female INAH-1) also produced a substantial bias in the Allen et al. [20] study material. However, the age distribution does not explain why LeVay [21] could not find a sex difference in the volume of INAH-1. However, cell numbers were not determined by this author.

Other Sexually Dimorphic Nuclei

Allen et al. [20] described two other cell groups (INAH-2 and -3) in the preoptic-anterior hypothalamic area that were larger in the male brain than in the female brain. LeVay [21] could not confirm the sex difference in INAH-2, but did find a difference in INAH-3. As immunocytochemistry was not performed, it is not clear whether the nuclei have to be considered as islands of the PVN or as separate anatomical entities. In addition, cell counts of the INAHs in the two sexes are lacking.

A clear sex difference was described by Allen and Gorski [22] in what they called the 'darkly staining posteromedial component of the bed nucleus of the stria terminalis (BNST-dspm)', an area in which the volume was found to be 2.5 times larger in males than in females. However, cell counts were not performed in this study, and therefore these findings remain preliminary.

The suprachiasmatic nucleus (SCN) stained with vasopressin as a marker, showed a sex difference in shape [23]. The shape of the SCN was elongated in women and more spherical in men. However, in this study no significant sex difference was observed in either volume, vasopressin cell number or total cell number of the SCN. It is not known whether this sex difference in shape correlates with sex differences in SCN afferent or efferent connections.

Only a few human brain structures in the two sexes have been investigated morphometrically, but as there is a sex difference in the relative size of the human brain [3], we expect that many more sex differences have yet to be revealed.

Development of the Human SDN

In mid-pregnancy the SDN can be distinguished in the human fetal brain [9, 24]. Yet the SDN cell number (fig. 4) and volume at term birth are only 22% and 18%, respectively, of the values found between 2 and 4 years of postnatal age. In the second half of gestation and during the first postnatal years there is no significant sex difference in the size of the SDN. During the first postnatal years the SDN cell number rapidly increases in both boys and girls up to the age of 2–4 years postnatally. Only after this age does the human SDN differentiate according to sex, due to a decrease in both SDN volume and cell number in women, whereas in men these parameters remain unaltered up to the age of about 50 years [9]. Our results do not support the proposition that gonadal hormones stimulate mitotic formation, migration or aggregation of SDN cells during the fetal or perinatal period. In mid-gestation and perinatally the levels of sex hormones are much higher in boys than in girls [1, 2]. Yet, the SDN size and cell number show the same magnitude in boys and girls up to the age of 2–4 years. The surprisingly late postnatal sexual differentiation of the human hypothalamus is in agreement with the observation that neither oestrogen, androgen nor progestin receptors were found in the human fetal brain at mid-gestation [25]. The sex difference in the volume of the BNST-dspm seems to occur only in adulthood, though there is reason for caution. The sample size of subjects between 10 and 20 years of age was small in the Allen and Gorski study [22]. Together, these data support, however, the notion that sexual differentiation of the human hypothalamus takes place after the

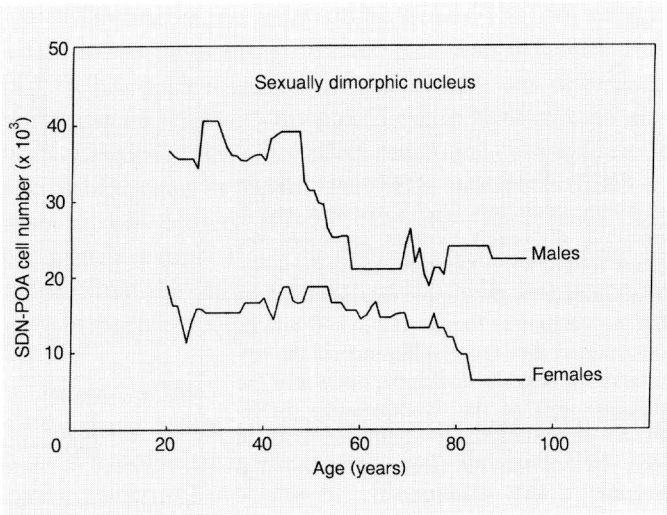


Fig. 3. Age-related changes in the total cell number of the sexually dimorphic nucleus (SDN) of the preoptic area (POA) in the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves. Note that in males SDN-POA cell number steeply declines between the age of 50 and 60 years, whereas in females, from the age of about 50 years, a more gradual cell loss is observed, which continues until 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 Hofman and Swaab [15] with permission.)

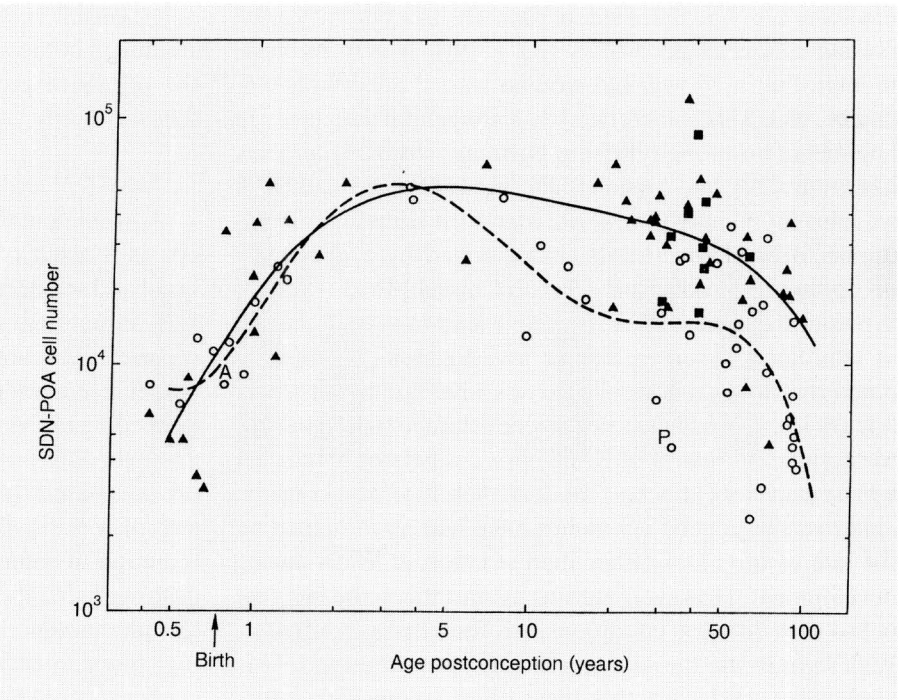


Fig. 4. Development and sexual differentiation of the human sexually dimorphic nucleus (SDN) of the preoptic area (POA) of the hypothalamus. Log-log scale. Note that at the moment of birth the SDN is equally small in boys (▲) and girls (○) and contains only about 20% of the cell number found at 2–4 years of age. The SDN cell number of a female neonate with a pituitary aplasia (A) is fully within the range of other neonates. Cell numbers reach a peak value at approximately 2–4 years postnatally, after which sexual differentiation occurs in the SDN. The cell number in the SDN of women decreases, whereas the cell number in men remains approximately unchanged until the age of 50 years. In women, cell number decreases for the second time after the age of about 60 years, following a period of relative stability, dropping to values which are only 10–15% of the cell numbers found at 2 years postnatally. Note that in men the reduction in cell number in senescence is less dramatic. The largest discrepancy in cell number between men and women is found at approximately 30 years of age and in people older than 80 years, whereas sexual dimorphism in the SDN cell number is least around the age of 60 years. The SDN cell number in homosexual men (■) does not differ from that in the male reference group. The cell number of the SDN of a woman with Prader-Willi syndrome (P) is small. The curves are quintic polynomial functions fitted to the original data for males (drawn line) and females (dashed line). (Adapted from Swaab and Hofman [9] with permission.)

perinatal period and before adulthood, rather than during mid-gestation. This, and our observation that sexual differentiation of the human SDN does not take place earlier than 4 years postnatally, calls for a re-evaluation of the possible relationship between the sex dimorphism of the SDN and the perinatal testosterone peak in boys, which lasts only some 90 days [26]. It is possible that the perinatal testosterone peak promotes cell survival a few years later by preventing the 'programmed' cell death which normally occurs in the female SDN. A similar mechanism is supposed to take place in rat spinal cord neurones of the bulbocavernosus musculature [8].

In addition, one may speculate that not only hormones, but also other chemical compounds and social factors, such as stress, might be involved in sexual differentiation of the brain in this period (compare [9, 27]). A case history of a 20-year-old man who had suffered a complete loss of testes at birth, showed a lack of sequelae in terms of gender identity, gender role and sexual functioning [28]. This observation may support the idea that factors other than androgens may be involved in sexual differentiation of the brain, or that the hormonal factors in the development of gender and sexual orientation exert their effects before birth.

SDN Development and Sex Hormones

Regarding the possible involvement of sex hormones in SDN development in humans, few data are available. Sources of information are the 'experiments of nature and

medicine'. A child that died, perinatally, of a pituitary aplasia had a normal sized SDN (fig.4; [9]). It is of interest, though, that a 30-year-old woman with Prader-Willi syndrome, which is characterized by a congenital deficiency in luteinizing hormone-releasing hormone (LHRH) and sex hormones [29], had a small SDN (fig.4; [9]). A small SDN was also found in a 57-year-old man with Klinefelter's syndrome, a condition that is characterized by a deficiency in androgen production [Swaab, unpublished results]. Whether these anomalies invariably lead to a smaller SDN in adulthood deserves further investigation. It must be noted that women normally do not have significant elevations of sex steroid levels in early development and that the endocrine differences of Klinefelter patients only become apparent in early puberty [30]. It is, therefore, also conceivable that the genetic anomalies have had an influence on the size of the SDN rather than sex steroid levels during development. However, arguing against this is the fact that in two such different conditions as Klinefelter's and Prader-Willi's syndrome the same anomalies were observed. One might also wonder whether these observations have implications for the development of sexual orientation. Vogt [31] did not find an indication for a higher incidence of homosexuality in his sample of 30 (46, XXY) Klinefelter patients. However, it must be noted that 15 men in his sample were sexually less active than normal males. This prevents any firm conclusion as to the sexual orientation of these subjects.

The Human Hypothalamus, Sexual Orientation and Gender Identity

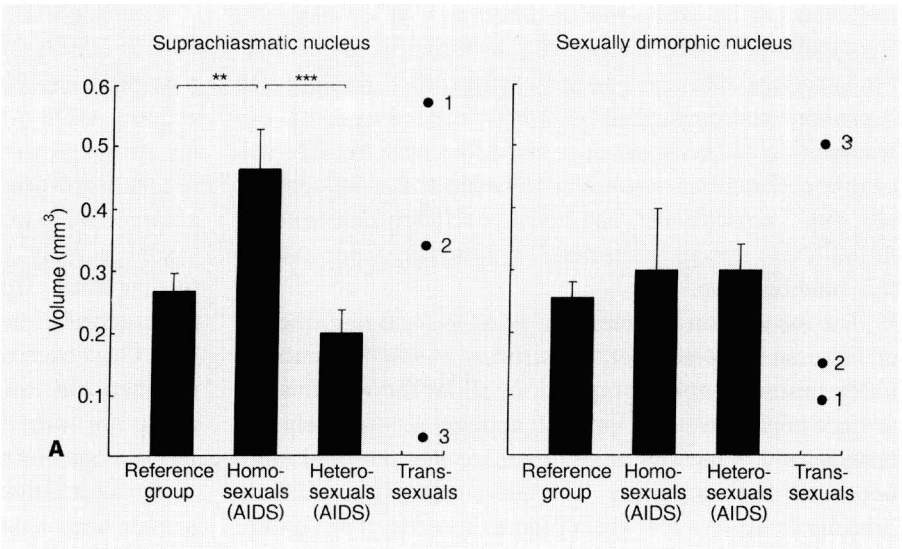
We had the opportunity to study the structure of the anterior hypothalamus in relation to sexual orientation, and investigated 34 subjects. Eighteen male subjects, 22–74 years of age, whose sexual orientation was generally not known, served as a reference group. The homosexual male group consisted of 10 non-demented AIDS subjects aged 25–43 years. Six non-demented heterosexuals (4 males, 2 females aged 21–73 years) who had also died of AIDS served as a control group. Two areas of the hypothalamus were studied: the SDN and the suprachiasmatic nucleus (SCN). The SCN is considered to be the principal component of the biological clock generating and coordinating hormonal, physiological and behavioural circadian rhythms [32–34]. In addition, the SCN is thought to be involved in reproduction, at least in laboratory animals [35, 36]. The main results were as follows: cell numbers in the SDN of the reference group, the male homosexuals and the heterosexual subjects did not differ. However, the SCN volume in homosexual men was 1.7 times as large as that of the reference group of male subjects and contained 2.1 times as many cells (fig.5).

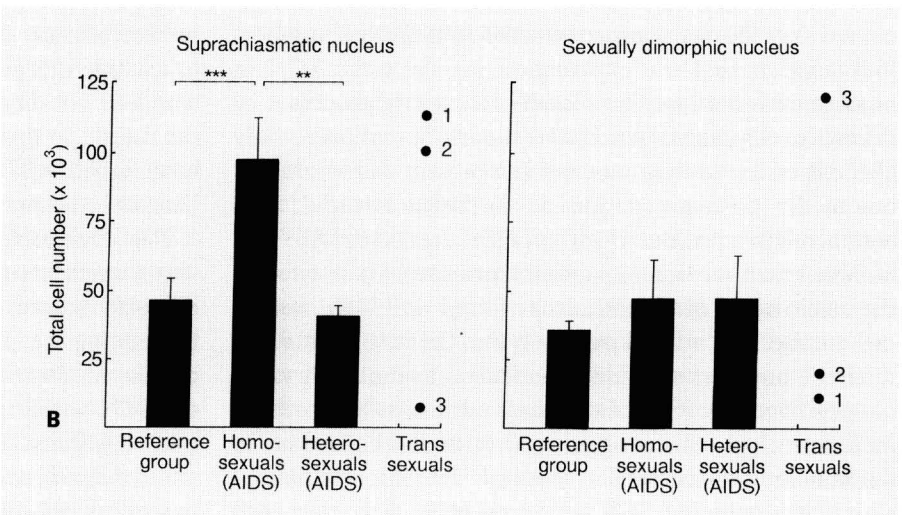
The SDN and Sexual Orientation

A prominent theory of sexual orientation is that it develops as a result of an interaction between the developing brain and sex hormones [6, 38–40]. A multitude of factors, such as maternal stress [11, 39, 41 but compare 42, 43] and chemicals [44] are thought to influence the process of sexual differentiation of the brain and sexual orientation. According to Dörner's hypothesis, male homosexuals have a female differentiation of the hypothalamus. This hypothesis was based solely on indirect evidence; the demonstration of a positive feedback of luteinizing hormone (LH) secretion in some homosexual men following injection of oestrogens [6, 45]. However, according to Gooren [46, 47], this phenomenon is probably related to changes in testicular function rather than to sexual orientation, and in his studies it could be demonstrated as often in homosexual as in heterosexual men. Furthermore, it could be shown that the same person was able to produce a negative and a positive oestrogen feedback effect, depending on the hormonal milieu. This demonstrated that it is not the organization of the hypothalamic-pituitary unit that determines the occurrence of a positive or negative oestrogen feedback. Also, it is not certain whether the rise in LH following oestrogen administration that Dörner [45] and Gladue et al. [6] observed, met the stringent endocrine criteria of a positive oestrogen feedback effect [48]. Dörner's hypothesis concerning sexual orientation became testable when we found that the SDN of the preoptic area of the human hypothalamus contains twice as many cells in men as in women [9, 14, 15]. In contrast to this hypothesis, neither the SDN volume nor the cell number in the hypothalamus of homosexual men (who died of AIDS) differed from that of the male reference group in the same age range (fig.5; [9]). More recent data (fig.5) confirmed and extended this observation with a heterosexual control group of subjects also suffering from AIDS [37]. The fact that no difference in SDN cell number was observed between homosexual and heterosexual men who died of AIDS refutes the global formulation of Dörner's hypothesis that male homosexuals have 'a female hypothalamus'.

The SCN and Sexual Orientation

The observation that the SCN in homosexual men contains 2.1 times as many cells as the SCN of the reference group (fig.5; [37]) implies that the difference in SCN vol-





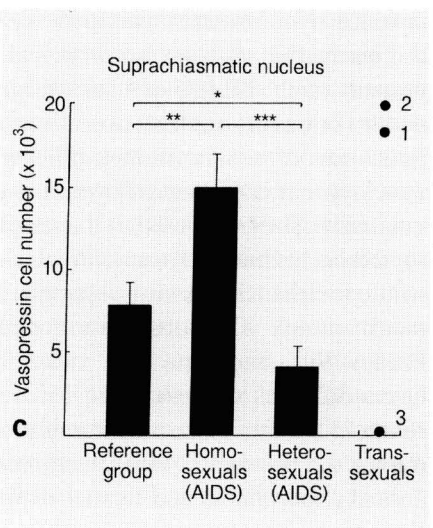


Fig. 5. A Volume of the human suprachiasmatic nucleus (SCN) and sexually dimorphic nucleus (SDN) as measured in four groups of adult subjects: (1) a male reference group (n = 18); (2) male homosexuals who died of AIDS (n = 10); (3) heterosexuals who died of AIDS (n = 6; 4 males and 2 females), and (4) three male-to-female transsexuals (numbers refer to T1–T3 in table 1). The values are medians and standard deviations. 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, *$p<0.05$; **$p<0.01$; ***$p<0.001$). Note that none of the parameters measured in the SDN (fig. 5A, B) showed significant differences among the three groups (p always >0.4). **B** Total number of cells in the human SCN and SDN. 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) neurones 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 neurones as the reference group of male subjects and 3.6 times as many VP neurones as the SCN in heterosexual AIDS patients. Notice that the SCN of heterosexual individuals who died of AIDS contains less VP cells than the subjects from the reference group. In the right-hand column of each figure, the individual values of three transsexual patients (T1–T3) are given. Note that 1 and 2 have a large SCN and a small SDN and that 3 has a small SCN and a large SDN. For clinical details see table 1. (Adapted from Swaab and Hofman [37] with permission.)

ume can not be attributed to differences in shrinkage of hypothalamic tissue during the histological procedure. However, the difference in SCN cell number in relation to sexual orientation can not be directly related to sexual differentiation of the brain since no differences in SCN volume or cell number were found between males and females [23, 49]. The possibility can not be excluded that sex hormone levels during brain development play some part in this phenomenon.

The association between a large SCN (and in particular an increase in the number of neurones) and male homosexuality raises a number of questions about the way this difference might have developed. It appears very unlikely that homosexual behaviour would increase the neuronal number in any brain structure. The nerve cells of the SCN are postmitotic from a few years of age onwards, if not earlier [50]. However, an increase in stainability of vasopressin neurones due to homosexual behaviour can not be excluded. Yet the developmental course of SCN cell numbers [50] suggests that the explanation for the large SCN in homosexual men may be found in the early processes of brain development. At birth, the SCN contains only 13–20% of the adult number of vasopressin and total cells, but in the postnatal period development is rapid. Cell counts reach a peak at 13–16 months after birth [50]. The SCN cell numbers found in adult homosexual men were of the same order of magnitude as found at 13–16 months postnatally. The normal pattern is that the vasopressin and total cell numbers subsequently decline to the adult value of approximately 35% of the peak values. In homosexual men, therefore, this programmed postnatal cell death in the SCN seems to have been reduced. The observation that a similarly enlarged SCN was present in a woman with Prader-Willi syndrome [36], a congenital LHRH deficiency in which sex hormone levels are very low [29] suggests that the interaction with sex hormones at some stage of development might be relevant for the programmed SCN cell death (however, see the caveat earlier in this text). The possibility of sex hormones playing some role in SCN development is reinforced by an observation of Södersten et al. [35]. They showed that the amplitude of the circadian rhythm in sexual behaviour, of which the SCN is the substrate, is enhanced by anti-oestrogen treatment of the neonatal animal. This observation and the large SCN in Prader-Willi syndrome [36] make it more likely that a larger SCN, as reported here for homosexual men, may relate to a difference in the interaction with sex hormones during development. This possibility should be tested in animal experiments and further explored in human material.

One might argue that the present finding of an enlarged SCN in male homosexuals who died of AIDS only holds for a particular subset of homosexual men (i.e. those likely to acquire AIDS through a high number of frequently changing sexual partners with whom anal receptive sexual techniques were performed [51, 52]. This possibility that an enlarged SCN may be related to, for example, the level of sexual activity rather than to homosexuality warrants further study. However, experiments in rats have shown a close correlation between sexual activity and SDN size [11]. Our observation that the size of the SDN in homosexual men did not differ from that of the male reference group nor from that of the heterosexual men who died of AIDS does not support this possibility.

An alternative explanation for the enlarged SCN found in male homosexuals who had died of AIDS is that it might be related to the hypogonadism in adulthood that has been found in AIDS patients [53]. Our observation that the SCN in heterosexual male AIDS patients is not enlarged seems to exclude this possible explanation, but homosexual men who had not died of AIDS should certainly be studied in the future. In this respect, it is interesting that we observed an enlarged SCN in two (primary) male-to-female transsexuals who did not suffer from AIDS [36] (fig.5).

The functional implications of the association between sexual orientation in men and SCN size are not clear. Various observations in animals suggest that the SCN, apart from being the biological clock, may be involved in reproductive processes [35, 36]. Judged from its nucleolar size, the SCN is also activated around puberty in rats [54]. In addition, lesions of the SCN area in the female rat attenuated the positive feedback response of gonadotrophic hormones to oestrogens [55, 56]. However, recently it was observed that lesions in the adult male rat SCN did not alter sexual orientation [F.H. De Jonge et al., unpubl. observ.]. This observation argues in favour of the possibility that sexual orientation and the size of the SCN are not causally related, but may be subject to the same organizing factor in development. The relationship between a large SCN and homosexuality is unexpected and difficult to interpret. The relationship need not be causal, in the sense that it is a necessary and sufficient condition for developing a homosexual orientation. It is imperative to study more material before definitive conclusions can be drawn. We have no information on the size of the SCN in female homosexuals or bisexuals. Until more data have been collected our finding is open to interpretation. It is particularly pertinent to study the SDN and SCN in subjects whose prenatal and postnatal history has been atypical (for example an excess of androgens in females or a deficiency or insensitivity to

Table 1. Results from the study of three transsexual subjects (T1, T2, T3)

	T1	T2	T3
Hypothalamus	Large SCN; small SDN	Large SCN; small SDN	Small SCN; large SDN
Male-to-female	Gender dysphoria from early childhood onwards	Gender dysphoria from early childhood onwards	Gender dysphoria postpubertally
Sexual/social orientation	Androphile, married under social pressure, father, last 4 years of life gynaephilic, hostile towards men	Asexual but surrounded herself with women	Gynaephilic, father following operation asexual
Hormone treatment	Cyproterone acetate, ethinylestradiol	Cyproterone acetate, stilbestrol ethinylestradiol	Cyproterone acetate, ethinylestradiol
Age at death (years)	50	44	43
Cause of death	Suicide	Probably cardiovascular	Sarcoma

androgens in males) as has been done in earlier work on sexual orientation (e.g. Money et al. [57]).

Transsexuality

It has been proposed that gender identity, like sexual orientation, develops as a result of an interaction between the developing brain and sex hormones. Transsexuality is considered to be the result of a disturbance of this interaction [6, 45]. In view of the similarity between the hypotheses on the development of gender and sexual orientation, it is of interest that 60% of male-to-female transsexuals are androphile and 10% are biphile. In 95% of cases, female-to-male transsexuals are gynaecophilic [58]. These data indicate that similar (but as yet unknown) mechanisms may play a role in the development of both gender and sexual orientation.

We were given the opportunity to study the hypothalami of three male-to-female transsexuals (fig.5, table 1). Two of them (T1, T2) appeared to have a large SCN with high cell numbers and a small SDN with low cell numbers. The third transsexual subject (T3) revealed exactly the opposite (i.e. a small SCN and a large SDN). These two different patterns could not be related to the sexual orientation of these three subjects (table 1) in any simple way. Table 1 suggests that a relationship might exist between a large SCN and small SDN and primary transsexuality (i.e. awareness of the gender problems from early childhood onwards) on the one hand, and a small SCN and large SDN and secondary transsexuality (i.e. awareness of transsexuality later in life) on the other. It will be obvious that more data are necessary in order to establish whether such a relationship exists.

Conclusions and Summary

Following observations in many mammalian species, the human hypothalamus is believed to undergo sexual differentiation during development, due to an organizing effect of sex hormones.

We have found a SDN in the preoptic area of the human hypothalamus that contains about twice as many cells in young adult men as in women. The magnitude of the sex difference in the SDN depends on age. In the literature two other hypothalamic nuclei (INAH-2 and -3) and a part of the bed nucleus of the stria terminalis have been reported to be sexually dimorphic in humans.

At term, the SDN contains only 20% of the cell number found at 2–4 years of age. The cell number rapidly increases in boys and girls, at the same rate, until 2–4 years of age, after which the SDN differentiates according to sex, due to a decrease in cell numbers in girls. This period of sexual differentiation of the human hypothalamus is much later than generally presumed in the literature, and reveals the possibility of postnatal factors such as hormones, other chemical compounds and psychosocial factors interacting in the sexual differentiation of the brain. A few preliminary observations in clinical conditions with deficient hormonal production suggest that the size and cell number of the human SDN in adulthood is influenced by sex hormones during development.

No difference in SDN cell number was observed between homosexual and heterosexual men. This refutes Dörner's hypothesis that male homosexuals have a 'female hypothalamus'.

In a sample of brains of homosexual men we found that the SCN contained twice as many cells as in the reference group. The observation that a similarly enlarged SCN was present in a woman with Prader-Willi syndrome suggests that sex hormones and SCN development might be interrelated. A recent report claimed INAH-3 to be more than twice as large in heterosexual men as in homosexual men. Preliminary data suggest that the SCN is large and the SDN small in primary male-to-female transsexuals and that the SCN is small and the SDN large in secondary male-to-female transsexuals, but more data have to be collected to confirm this observation.

In conclusion, differences in size and cell number have been reported in a number of hypothalamic nuclei in relation to sexual orientation and gender. However, the functional implications of these findings are far from clear.

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