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An enlarged suprachiasmatic nucleus in homosexual men

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Morphometric analysis of the human hypothalamus revealed that the volume of the suprachiasmatic nucleus (SCN) in homosexual men is 1.7 times as large as that of a reference group of male subjects and contains 2.1 times as many cells. In another hypothalamic nucleus which is located in the immediate vicinity of the SCN, the sexually dimorphic nucleus (SDN), no such differences in either volume or cell number were found. The SDN data indicate the selectivity of the enlarged SCN in homosexual men, but do not support the hypothesis that homosexual men have a 'female hypothalamus'.

INTRODUCTION

The suprachiasmatic nucleus (SCN) of the hypothalamus is a cell group located in the basal part of the mammalian brain (Fig. 1). It is considered to be the principal component of the biological clock generating and coordinating hormonal, physiological and behavioral circadian rhythms[24,25,29]. In addition it is thought to be involved in reproduction[30,33]. Because of the differences in circadian rhythms found in relation to sex[11,42] and the attenuation of circadian rhythmicity with aging as well as in Alzheimer's disease[5,28,41] the human SCN has been studied with particular reference to these conditions[21,32,33]. We found, for example, a marked cell loss in the human SCN in late onset Alzheimer's disease[32,33] dropping to values which were only about 30% of the cell number found in normal adults.

In order to investigate whether cell loss in the SCN also occurs in other types of dementias, we subsequently examined the SCN in postmortem brains of subjects with early onset Alzheimer's disease as well as of patients who died with an AIDS–dementia complex[27]. Our studies revealed that the SCN indeed was as strongly affected in early onset as in late onset Alzheimer's disease[19]. However, we found that the SCN was not smaller in subjects with AIDS–dementia complex than in the reference group, but rather appeared to be considerably enlarged. Subsequent research, as reported in the present paper, suggested that the enlarged SCN is related neither to the AIDS–dementia complex nor to AIDS per se but rather to homosexuality.

MATERIALS AND METHODS

Subjects

For the present study the brains of 34 subjects were investigated (Table I). The required, separate permission for brain autopsy was obtained either from the patients themselves or, in case of dementia, from partners or relatives. The reference group consisted of brains of 18 male subjects from 22 to 74 years of age (39.9 ± 3.6 years; mean ± S.E.M.). General pathology and neuropathology were performed either at the Free University of Amsterdam (Dr. W. Kamphorst) or at the Academic Medical Center of the University of Amsterdam (Dr. D. Troost). Sexual preference of the subjects of the reference group was generally not known. The homosexual male group consisted of 10 non-demented AIDS subjects (aged 25–43; 36.7 ± 2.1 years). AIDS patients were diagnosed according to the Centers for Disease Control[6]. As a control group the same parameters were measured in 6 non-demented heterosexuals (4 males, 2 females; aged 21–73 years; 36.7 ± 7.5 years) who also died from AIDS. Two contracted AIDS by blood transfusion, two by sexual contact and two were drug addicts. Excluding the two female subjects from the group of heterosexuals with AIDS, in order to confine the analysis exclusively to male subjects, did not in any way affect the outcomes. Sexual preference of the subjects of the homosexual and heterosexual AIDS groups was registered in the clinical records. Only after the measurements were performed, was it established from the records whether or not the AIDS patients belonged either to the non-demented or demented homosexual groups or to the heterosexual group.

Histology

Brains were generally weighed after removal followed by fixation in formaldehyde at room temperature. No significant differences in postmortem delay were found among the 3 groups considered ($P > 0.2$). In contrast, the fixation times in the reference group were on average longer than in the homosexual male group (40 ± 3 days and 29 ± 4 days, respectively, $P < 0.05$). Since the length of the fixation period and the SCN volume and cell number were not found to be correlated ($P > 0.3$) the difference in fixation time between these groups will not affect the outcome. After fixation, the hypothalamic area was dissected, dehydrated and embedded in paraffin. Serial 6

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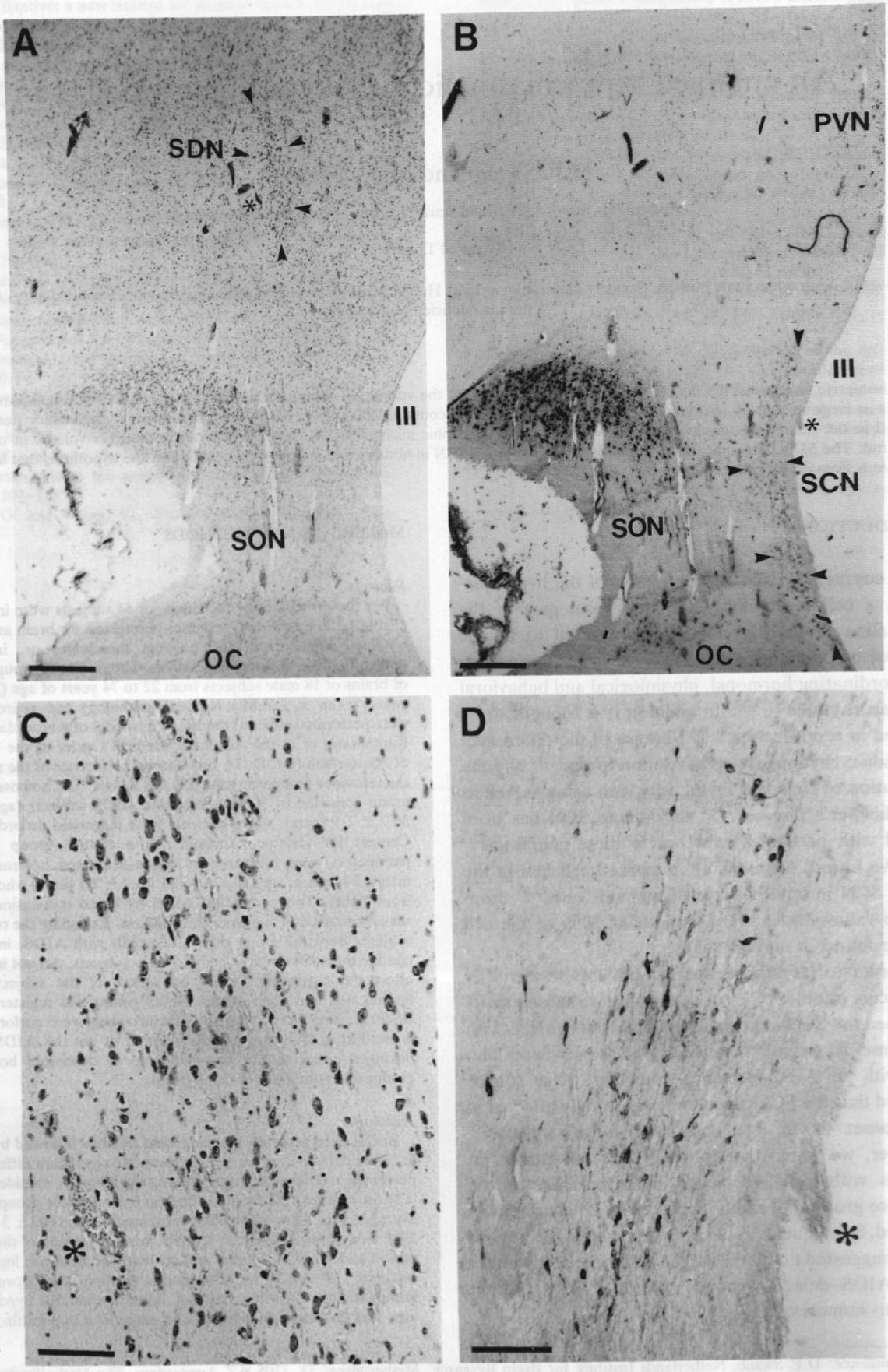
A
SDN
III
SON
OC
B
PVN
III
SCN
SON
OC
C
D

TABLE I

Brain material

All patients were male subjects, except for two female patients in the group of heterosexuals who died from AIDS (patient nos. 33 and 34).

Patient number	*Age (years)*	*Brain weight (g)**	*Suprachiasmatic nucleus*			*Postmortem delay (h)*	*Fixation (days)*	*Clinical diagnosis*
			Volume (mm^3)	*Total cell number ($\times 10^3$)*	*AVP cell number ($\times 10^3$)*			
Reference group								
1	22	1570	0.380	46.82	12.93	15	35	Lymphatic leukemia
2	23	1310	0.379	58.98	9.92	13	11	Encephalitis in brainstem
3	27	1330	0.129	17.75	3.45	40	28	Coma
4	27	1560	0.196	22.87	3.97	24	40	Drug addiction; sepsis (*Staphylococcus aureus*)
5	28	1510	0.303	40.69	5.45	23	32	Medial cerebral artery aneurysm; vena cave superior syndrome; lung emboli
6	28	1450	0.233	43.27	9.89	24	46	Guillain-Barré syndrome
7	29	1400	0.279	60.51	5.24	13	41	Congenital heart disease; cardiac failure
8	31	1330	0.339	74.99	9.25	29	30	Multiple trauma; small subarachnoidal hemorrhage
9	37	1370	0.133	18.76	1.87	39	35	Bronchopneumonia
10	37	1510	0.169	24.79	2.95	48	46	Alcohol intoxication, combined with benzodiazepines
11	41	1440	0.464	84.55	13.73	120	44	Cerebral contusion, lung emboli
12	42	1510	0.214	54.31	9.60	22	42	Metastatic bronchogenic carcinoma; pneumothorax
13	43	1260	0.195	58.93	10.75	23	53	Non-Hodgkin lymphoma; sepsis
14	47	1620	0.138	24.02	2.24	24	39	Amyotrophic lateral sclerosis/spinal muscular atrophy
15	59	1350	0.260	33.77	6.35	4	53	Pulmonary emphysema; pneumothorax
16	61	1400	0.285	56.97	8.10	22	51	Myocardial infarction; cardiac failure
17	63	1420	0.305	53.38	8.93	32	35	Myocardial infarction; cardiac failure
18	74	1410	0.246	37.00	7.34	13	48	Cardiac failure; bronchopneumonia
Homosexuals (AIDS)								
19	25	1530	0.534	106.75	18.40	47	28	AIDS, pneumonia
20	30	1480	0.669	101.29	21.80	4	31	AIDS, cytomegalic infections
21	30	1640	0.413	59.11	15.57	24	26	AIDS, *Pneumocystis carinii* pneumonia
22	32	1440	0.473	99.94	12.26	49	11	AIDS, *Pneumocystis carinii* pneumonia
23	39	>1320	0.432	117.59	10.83	24	28	AIDS, progressive multifocal leukoencephalopathy
24	41	>1240	0.634	147.73	20.61	12	34	AIDS, bronchopneumonia, cytomegalic infections and toxoplasmosis
25	42	1340	0.109	24.66	2.70	4	35	AIDS, disseminated Karposi sarcoma and generalized mycobacterium avium infection
26	42	1340	0.481	90.91	12.15	19	30	AIDS, cytomegalic meningoencephalitis
27	43	>1260	0.279	56.47	16.94	2	96	AIDS, disseminated Karposi sarcoma and pneumonia
28	43	>1340	0.375	64.20	10.29	24	17	AIDS, *Pneumocystis carinii* pneumonia, Karposi sarcomas, cytomegalic infections
Heterosexuals (AIDS)								
29	21	1500	0.154	36.96	3.35	17	26	AIDS, mycobacterial infections pneumonia, cerebrovascular accident
30	30	1430	0.092	23.82	2.56	8	35	AIDS, *Pneumocystis carinii* pneumonia, lung tuberculosis, toxoplasmosis, heroin addiction
31	30	1340	0.244	40.77	6.97	8	26	AIDS, disseminated non-Hodgkin lymphoma infections, drug use
32	32	1340	0.128	43.56	1.48	11	131	AIDS, cytomegalic infections
33	34	1400	0.181	40.38	5.14	12	24	AIDS, disseminated histoplasmosis
34	73	>1090	0.258	55.88	4.38	48	38	AIDS, pneumonia, epilepsia

* Inequality signs indicate that parts of the brain were already removed directly after autopsy before weighing the brain.

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Fig. 1. Thionin staining (A,C) and vasopressin staining (B,D) of the 6 μm hypothalamus adjacent sections of patient no. 28. In the overview the SDN (A) and SCN (B) are indicated by arrowheads. The asterisk marks the site of a blood vessel and ventricular wall that corresponds with the asterisk in the higher magnification (C and D, respectively). The bars indicate 1 mm (A and B) or 0.1 mm (C and D). OC, optic chiasm; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SDN, sexually dimorphic nucleus of the preoptic area; SON, supraoptic nucleus; III, third ventricle.

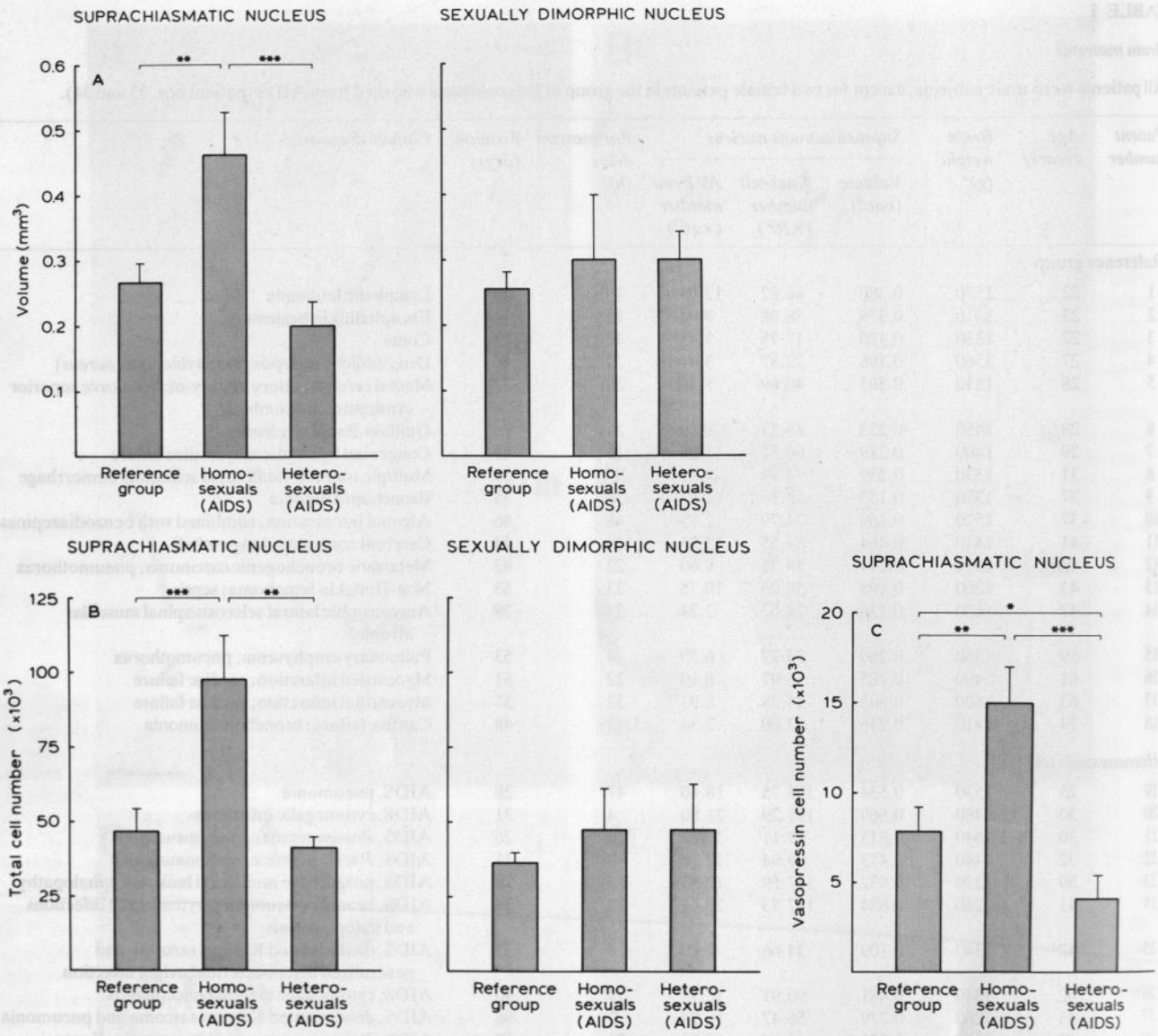

Fig. 2. A: volume of the human SCN and SDN as measured in 3 groups of adult subjects: (1) a male reference group (n = 18); (2) male homosexuals who died from AIDS (n = 10); and (3) heterosexuals who died from AIDS (n = 6; 4 males and 2 females). The values indicate medians and the standard deviation of the median[23]. 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. 2A,B) showed significant differences among the 3 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 neurons in the human SCN (the human SDN does not contain vasopressin-producing cells[22,31,34]. The SCN in homosexual men contains, on average, 1.9 times as many vasopressin-(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. Notice that the SCN of heterosexual individuals who died from AIDS, contains less vasopressin cells than the subjects from the reference group.

μm frontal sections were cut on a Leitz microtome, mounted on chrome-alum-coated slides, hydrated, brought to phosphate-buffered saline (PBS) and each 50th section was stained with thionin for orientation. Volume and cell numbers were determined in two hypothalamic nuclei, the suprachiasmatic nucleus (SCN) and the sexually dimorphic nucleus (SDN)[22,32,34]. The SDN (Fig. 1), which is identical with the intermediate nucleus[3] and twice as large in males as in females, was included as a reference nucleus in order to test the hypothesis of 'female differentiation of the hypothalamus' as a biological explanation for male homosexuality[13,15]. The SDN was visualized in thionin-stained sections[31] (Fig. 1) whereas for the SCN vasopressin — one of the main neurotransmitters or neuromodulators in this structure — was used as a marker[32] (Fig. 1).

Immunocytochemistry

For immunocytochemistry the hydrated sections were rinsed in PBS, pH 7.4 for 10 min, after which they were: (1) incubated with anti-AVP (Truus, 18/9/85) 1:800 in 0.5% Triton in PBS overnight at 4 °C; (2) washed in PBS (2 × 10 min); (3) incubated with goat anti-rabbit serum (Betsie) 1:50 in PBS for 30 min; (4) washed in PBS (2 × 10 min); (5) incubated with peroxidase–antiperoxidase (PAP) 1:500 to 1:1000 for 30 min; (6) washed in PBS (2 × 10 min); (7)

rinsed in 0.05 M Tris-HCl (Merck), pH 7.6; (8) incubated in 0.05 mg/ml 3,3-diaminobenzidine (Sigma) in 0.05 M Tris-HCl, pH 7.6, 0.01% H_2O_2 (Merck) for 10 min; (9) washed in aqua dest; (10) dehydrated in ethanol and mounted in Entellan. In the sections stained with anti-vasopressin, the borders of the SCN can be delineated reliably[32] (Fig. 1).

Morphometry

Area measurements of the vasopressinergic SCN and its cell nuclei were performed unilaterally by means of a digitizer (Calcomp 2000) connected to a VAX 11/780, using a Zeiss microscope equipped with 10× and 40× (PLAN) objectives, respectively, and with 12.5× (PLAN) oculars. In order to describe the shape of this nucleus[32] the rostrocaudal axis, the maximal cross-sectional area covered by vasopressin cells and fibers and the SCN volume were determined. The rostrocaudal axis was determined by staining every 25th section with anti-vasopressin starting from the lamina terminalis and ending at the caudal end of the optic chiasm. The rostral and caudal borders of the SCN were assessed by staining every 10th section in the area, and by determining the sections in which, respectively, the first and the last vasopressin cells were present. The maximal cross-sectional SCN area covered with vasopressin cells in the rostrocaudal series was determined as a separate parameter. The volume of the SCN was determined by integrating all the area measurements[37] of the SCN sections that contained immunocytochemically stained cells.

In addition, the vasopressin and total cell number were determined in the SCN of each subject. The number of vasopressin cells per unit SCN volume was estimated using a discrete 'unfolding' procedure[39], which included the modification proposed by Cruz-Orive[9] and a correction for section thickness (6 μm). The total SCN cell number was estimated by counting the profile density per unit area in thionin-counterstained material by means of the same procedure. For this purpose the section with the maximal SCN area was selected and nuclear profiles were determined per subject. The computer programs for these procedures were developed by Dr. R.W.H. Verwer at our Institute (for details, see ref. 32).

The volume and total cell number of the SDN were measured in the same way at the same side of the brain in thionin-stained sections (for details, see ref. 31).

Differences among the groups were tested two-tailed using the Kruskal–Wallis multiple comparisons test statistic[7]. Throughout this study values are expressed as medians ± the standard deviation of the median. The critical level for statistical significance was taken to be 5%.

RESULTS

The SCN volume in homosexual males was 1.73 times larger than in the male subjects of the reference group (0.463 ± 0.066 mm^3 and 0.267 ± 0.030 mm^3, respectively; $P < 0.01$) (Fig. 2A) and contained 2.09 times as many cells (97.5 ± 14.6 × 10^3 and 46.7 ± 7.6 × 10^3, respectively; $P < 0.001$) (Fig. 2B). Similar differences between these two groups were found for the number of vasopressin neurons (15.0 ± 2.1 × 10^3 and 7.9 ± 1.3 × 10^3, respectively; $P < 0.01$) (Fig. 2C). In addition, the rostrocaudal axis of the SCN was longer in homosexual males than in the male subjects of the reference group (2.37 ± 0.35 mm and 1.47 ± 0.42 mm, respectively; $P < 0.02$), whereas no such differences were found in the maximal cross-sectional area of the nucleus. In other words, the enlarged volume of the SCN in male homosexuals is mainly due to an extension of this nucleus in rostrocaudal direction, as a result of which homosexuals have a more elongated SCN than heterosexuals. Because the SCN in heterosexual patients who died from AIDS, was not significantly different in volume or total cell number from that of the reference group, an enlarged SCN seems not to be related to the terminal course of illness or to AIDS (Table I) but rather to homosexuality. Since the number of vasopressin neurons was smaller in the heterosexual AIDS group than in the reference group ($P < 0.05$) (Fig. 2C), while the total cell number was unaltered (Fig. 2B), AIDS seems to be accompanied by a reduction in the number of neurons expressing vasopressin. This might imply that the number of vasopressin neurons in homosexual men without AIDS may even be higher than observed in Fig. 2. Cell numbers in the SDN of the reference group, the male homosexuals and the heterosexual subjects did not differ significantly implying some degree of selectivity for the SCN enlargement in the hypothalamus of homosexual men.

DISCUSSION

The prominent theory on sexual orientation, i.e. that it develops as a result of an interaction between the developing brain and sex hormones[13,15] does not seem to be supported by our data on the SDN. Maternal stress[14] or chemicals[34] are thought to influence the process of sexual differentiation of the brain. According to Dörner's hypothesis, male homosexuals would have a female differentiation of the hypothalamus. This hypothesis was, however, sofar based solely on indirect evidence, i.e. the existence of a positive feedback on luteinizing hormone secretion in some homosexual men following injection of estrogens[13,15]. However, according to Gooren[16,17], this phenomenon is probably related to changes in testicular function rather than to sexual orientation and in his studies could be demonstrated as often in homosexual as in heterosexual men. Dörner's hypothesis became directly 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[22,31,34]. The SDN was first described in the rat[18] where it appears to be involved in male sexual behavior[2,12,36]. Neither the SDN volume nor the cell number in the hypothalamus of homosexual men who died from AIDS, however, differed from that of the male reference group in the same age range[34]. The present data confirm and extend this observation with a heterosexual control group of subjects also suffering from AIDS. The fact that no difference in SDN cell number was observed between homo- and heterosexual men who died from AIDS ($P = 0.50$) refutes the most global formulation of Dörner's hypothesis that male homosexuals have 'a female brain'.

The present data revealed that the volume of the SCN in homosexual men is 1.7 times as large as that of a reference group. Since the SCN in the former group also contains 2.1 times as many cells, this difference cannot be attributed to differences in shrinkage. The difference in SCN cell number in relation to sexual orientation can, however, 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[21,32]. The possibility cannot be excluded, yet, that sex hormone levels during brain development do play some part in this phenomenon (see below).

The association between a large SCN (and, in particular, an increase in the number of neurons) and male homosexuality raises a number of questions about the way it might have arisen. It appears very unlikely that homosexual behavior 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[35]. An increase in stainability of vasopressin neurons due to homosexual behavior is also unlikely, since the vasopressin cell densities do not differ among the 3 groups ($P > 0.2$). Although such a functional interpretation of the data cannot be totally excluded, the development of SCN cell numbers suggests that the explanation for the large SCN in homosexual men most likely may be found in early 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 around 13–16 months after birth[35] and are then of a similar magnitude as in adult homosexual men. In the reference group, the vasopressin and total cell numbers subsequently decline to the adult value of about 35% of the peak values. In homosexual men, therefore, this programmed postnatal cell death in the SCN seems to be limited. The observation that a similarly enlarged SCN was present in a woman with Prader-Willi syndrome[33], a congenital luteinizing hormone-releasing hormone deficiency in which sex hormone levels are very low[4], suggests that the interaction with sex hormones in some stage of development might be essential for the programmed SCN cell death. The possibility of sex hormones playing some role in SCN development is reinforced by an observation of Södersten et al.[30]. They showed that the amplitude of the daily rhythm in sexual behavior, for which the SCN is responsible, is enhanced by anti-estrogen treatment of the neonatal animal. This observation and the large SCN in Prader-Willi syndrome[33] suggests 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.

Dementia as a result of AIDS[26] does not seem to affect the size and cell number of the SCN in a significant manner, as can be concluded from comparing a group of 4 demented homosexual patients who died from AIDS, with the group of 10 non-demented homosexual AIDS patients from the present study. However, because of the dementia, these 4 cases were not included in the present study.

One might argue that the present finding of an enlarged SCN in male homosexuals who died from AIDS, holds only for a particular subset of homosexual men, i.e. those with a high number of frequently changing sexual partners with whom anal receptive sexual techniques were performed[10,38]. This possibility, i.e. that an enlarged SCN may be related to, e.g. the level of sexual activity rather than to homosexuality certainly warrants further study. Experiments in rats, however, have shown a close correlation between sexual activity and SDN size[2]. 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 that died from AIDS, does not support this possibility.

An alternative explanation for the enlarged SCN found in male homosexuals is that it might be related to hypogonadism in adulthood, as has been found in AIDS patients[8]. The observation that the SCN in heterosexual male AIDS patients is not enlarged seems to exclude this possible explanation, but homosexual men who did not die of AIDS should certainly be studied in the future. In this respect, it is interesting that we observed an enlarged SCN in two male-to-female transsexuals who did not suffer from AIDS[33].

The conclusion of the present paper is that the SDN data do not support the global hypothesis that homosexual men have a 'female brain'[13,15]. An association was found, however, between sexual orientation in men and SCN size, from which the functional implications are momentarily not clear. However, various observations in animals suggest that the SCN, apart from being the biological clock, may be involved in reproductive processes[30,33]. The SCN is also activated around puberty[1]. In addition, lesions of the SCN area in the female rat attenuated positive feedback response of gonadotropic hormones to estrogens[20,40]. The relationship between a large SCN and homosexuality is, of course, not necessarily a causal one. Animal experimental research has to reveal whether the SCN is causally implicated in sexual orientation, or whether SCN size and sexual preference are influenced by a common factor during development.

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REFERENCES

1 Anderson, C.H., Nucleolus: changes at puberty in neurons of the suprachiasmatic nucleus and the preoptic area, *Exp. Neurol.*, 74 (1981) 780–786.
2 Anderson, R.H., Fleming, D.E., Rhees, R.W. and Kinghorn, E., Relationships between sexual activity, plasma testosterone, and the volume of the sexually dimorphic nucleus of the preoptic area in prenatally stressed and non-stressed rats, *Brain Research*, 370 (1986) 1–10.
3 Braak, H. and Braak, E., The hypothalamus of the human adult: chiasmatic region, *Anat. Embryol.*, 176 (1987) 315–330.
4 Bray, G.A., Dahms, W.T., Swerdloff, R.S., Atkinson, R.L. and Carrel, R.E., The Prader-Willi syndrome: a study of 40 patients and a review of the literature, *Medicine*, 62 (1983) 59–80.
5 Bremner, W.J., Vitiello, M.V. and Prinz, P.N., Loss of circadian rhythmicity in blood testosterone levels with aging in normal men, *J. Clin. Endocrinol. Metab.*, 24 (1983) 1278–1281.
6 Centers for Disease Control, Classification system for HTLV-III/LAV infections, *MMWR*, 35 (1986) 334–339.
7 Conover, W.J., *Practical Non-parametric Statistics*, 2nd edn., Wiley, New York, 1980.
8 Croxson, T.S., Chapman, W.E., Miller, L.K., Levit, C.D., Senie, R. and Zumoff, B., Changes in the hypothalamic–pituitary–gonadal axis in human immunodeficiency virus-infected homosexual men, *J. Clin. Endocrinol. Metab.*, 68 (1989) 317–321.
9 Cruz-Orive, L.M., Particle shape-size distributions: the general spheroid problem. II. Stochastic model and practical guide, *J. Micr.*, 112 (1978) 153–167.
10 Curran, J.W., Meade Morgan, W., Hardy, A.M., Jaffe, H.W., Darrow, W.W. and Dowdle, W.R., The epidemiology of AIDS: current status and future prospects, *Science*, 229 (1985) 1352–1357.
11 Davis, F.C., Development of the suprachiasmatic nuclei and other circadian pacemakers. In D.C. Klein (Ed.), *Melatonin Rhythm Generating System*, Karger, Basel, 1982, pp. 1–19.
12 De Jonge, F.H., Louwerse, A.L., Ooms, M.P., Evers, P. and Van de Poll, N.E., Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats, *Brain Res. Bull.*, 23 (1989) 483–492.
13 Dörner, G., Neuroendocrine response to estrogen and brain differentiation in heterosexuals, homosexuals, and transsexuals, *Arch. Sexual Behav.*, 17 (1988) 57–75.
14 Ellis, L., Ames, M.A., Peckham, W. and Burke, D., Sexual orientation of human offspring may be altered by severe maternal stress during pregnancy, *J. Sex Res.*, 25 (1988) 152–157.
15 Gladue, B.A., Green, R. and Helleman, R.E., Neuroendocrine response to estrogen and sexual orientation, *Science*, 225 (1984) 1496–1499.
16 Gooren, L.J.G., The neuroendocrine response of luteinizing hormone to estrogen administration in the human is not sex specific but dependent on the hormonal environment, *J. Clin. Endocrinol. Metab.*, 63 (1986) 583–588.
17 Gooren, L.J.G., The neuroendocrine response of luteinizing hormone to estrogen administration in heterosexual, homosexual, and transsexual subjects, *J. Clin. Endocrinol. Metab.*, 63 (1986) 589–593.
18 Gorski, R.A., Gordon, J.H., Shryne, J.E. and Southam, A.M., Evidence for a morphological sex difference within the medial preoptic area of the rat brain, *Brain Research*, 148 (1978) 333–346.
19 Goudsmit, E., Hofman, M.A., Fliers, E. and Swaab, D.F., The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease, *Neurobiol. Aging*, 11 (1990) 529–536.
20 Gray, G.D., Södersten, P., Tallentrie, D. and Davidson, J.M., Effects of lesions in various structures of the suprachiasmatic-preoptic region on LH regulation and sexual behavior in female rats, *Neuroendocrinology*, 25 (1978) 174–191.
21 Hofman, M.A., Fliers, E., Goudsmit, E. and Swaab, D.F., Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain, *J. Anat.*, 160 (1988) 127–143.
22 Hofman, M.A. and Swaab, D.F., The sexually dimorphic nucleus of the preoptic area in the human brain: a comparative morphometric study, *J. Anat.*, 164 (1989) 55–72.
23 McGill, R., Tukey, J.W. and Larsen, W.A., Variations of box plots, *Amer. Stat.*, 32 (1978) 12–16.
24 Moore, R.Y., Central neural control of circadian rhythms. In W.F. Ganong and L. Martini (Eds.), *Frontiers in Neuroendocrinology, Vol. 5*, Raven, New York, 1978, pp. 185–206.
25 Moore-Ede, M.C., Sulzman, F.M. and Fuller, C.A., *The Clocks that Time Us. Physiology of the Circadian Timing System*, Harvard Univ. Press, Cambridge, MA, 1982.
26 Portegies, P., De Gans, J., Lange, J.M.A., Derix, M.M.A., Speelman, H., Bakker, M., Danner, S.A. and Goudsmit, J., Declining incidence of AIDS dementia complex after introduction of zidovudine treatment, *Br. Med. J.*, 299 (1989) 819–821.
27 Price, R.W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A.C. and Cleary, P., The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex, *Science*, 239 (1988) 586–592.
28 Prinz, P.N., Vitaliano, P.P., Vitiello, M.V., Bokan, J., Raskind, M., Perskind, E. and Gerber, C., Sleep, EEG and mental function changes in senile dementia of the Alzheimer's type, *Neurobiol. Aging*, 3 (1982) 361–370.
29 Rusak, B. and Zucker, I., Neural regulation of circadian rhythms, *Physiol. Rev.*, 59 (1979) 449–526.
30 Södersten, P., Hansen, S. and Srebro, B., Suprachiasmatic lesions disrupt the daily rhythmicity in the sexual behaviour of normal male rats and of male rats treated neonatally with antioestrogen, *J. Endocrinol.*, 88 (1981) 125.
31 Swaab, D.F. and Fliers, E., A sexually dimorphic nucleus in the human brain, *Science*, 228 (1985) 1112–1115.
32 Swaab, D.F., Fliers, E. and Partiman, T.S., The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia, *Brain Research*, 342 (1985) 37–44.
33 Swaab, D.F., Roozendaal, B., Ravid, R., Velis, D.N., Gooren, L. and Williams, R.S., Suprachiasmatic nucleus in aging, Alzheimer's disease, transsexuality and Prader-Willi syndrome. In E.R. de Kloet, V.M. Wiegant and D. de Wied (Eds.), *Neuropeptides and Brain Function, Progr. Brain Res., Vol. 72*, Elsevier, Amsterdam, 1987, pp. 301–311.
34 Swaab, D.F. and Hofman, M.A., Sexual differentiation of the human hypothalamus: ontogeny of the sexually dimorphic nucleus of the preoptic area, *Develop. Brain Res.*, 44 (1988) 314–318.
35 Swaab, D.F., Hofman, M.A. and Honnebier, M.B.O.M., Development of vasopressin neurons in the human suprachiasmatic nucleus in relation to birth, *Develop. Brain Res.*, 52 (1990) 289–293.
36 Turkenburg, J.L., Swaab, D.F., Endert, E., Louwerse, A.L. and Van de Poll, N.E., Effects of lesions of the sexually dimorphic nucleus on sexual behavior of testosterone-treated female Wistar rats, *Brain Res. Bull.*, 21 (1988) 215–224.
37 Van Eden, C.G., Uylings, H.B.M. and Van Pelt, J., Sex difference and left–right asymmetries in the prefrontal cortex

during postnatal development in the rat, *Develop. Brain Res.*, 12 (1983) 146–153.
38 Van Griensven, G.J.P., Tielman, R.A.P., Goudsmit, J., v.d. Noordaa, J., De Wolf, F., De Vroome, E.M.M. and Coutinho, R.A., Risk factors and prevalence of HIV antibodies in homosexual men in The Netherlands, *Am. J. Epidem.*, 125 (1987) 1048–1057.
39 Weibel, E.R., *Stereological Methods, Vol. 1. Practical Methods for Biological Morphometry*, Academic, London, 1979.
40 Wiegand, S.J., Terasawa, E., Bridson, W.E. and Gray, R.W., Effects of discrete lesions of preoptic and suprachiasmatic structures in the female rat: alterations in the feedback regulation of gonadotropin secretion, *Neuroendocrinology*, 31 (1980) 147–157.
41 Witting, W., Kwa, I.H., Eikelenboom, P., Mirmiran, M. and Swaab, D.F., Alterations in the circadian rest–activity rhythm in aging and Alzheimer's disease, *Biol. Psychiatr.*, 27 (1990) 563–572.
42 Yamaoka, S., Modification of the circadian sleep rhythms by gonadal steroids and the neural mechanisms involved, *Brain Research*, 185 (1980) 385–398.