

The sexually dimorphic nucleus of the preoptic area in the human brain: a comparative morphometric study

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INTRODUCTION

In mammals the preoptic-anterior region of the hypothalamus (POA/AH) is thought to be implicated in the neural control of endocrine functions (Kelley & Pfaff, 1987). Particularly the medial preoptic area is critically involved in the regulation of sexually differentiated functions under the influence of gonadal steroids (for references see Anderson, Fleming, Rhees & Kinghorn, 1986; Watson, Hoffman & Wiegand, 1986). In addition, the POA/AH is considered to be the principal brain structure in the mammalian central clock mechanism, implicated in the generation and/or co-ordination of a variety of biological rhythms (e.g. Moore, 1978; Rusak & Zucker, 1979; Moore-Ede, Sulzman & Fuller, 1982).

Recent studies have attempted to identify sex differences in the structure of the POA/AH that may underline these functional differences (see De Vries, De Bruin, Uylings & Corner, 1984). Sexual differences in the gross morphology of the *preoptic area*, as well as in its ultrastructure, have been reported in the rat, mouse, hamster, gerbil, guinea-pig, ferret, monkey and man (see Robinson, Fox, Dikkes & Pearlstein, 1986). Recently, the existence of an intensely staining cell group within this area has been demonstrated, the size of which shows a markedly sexual dimorphism. Described first by Gorski and his co-workers in the rat (Gorski, Gordon, Shryne & Southam, 1978) this sexually dimorphic nucleus of the preoptic area (SDN-POA) is still the most conspicuous morphological sex difference in the mammalian brain. It is possible, in fact, to determine the sex of a rat by direct visual observation of Nissl-stained histological sections (Gorski *et al.* 1980). In the male rat, in which the SDN-POA is 3–8 times larger than that of the female, this difference has been shown to be independent of the steroidal environment in the adult, but it may be profoundly influenced by the perinatal steroidal environment (Jacobson, Shryne, Shapiro & Gorski, 1980). Although the SDN-POA has been studied most extensively in the rat (see also Hsü, Chen & Peng, 1980; Anderson, Rhees & Fleming, 1985; Robinson *et al.* 1986), similar sexual differences have been documented in homologous structures in guinea-pigs (Hines *et al.* 1985; Byne & Bleier, 1987), ferrets (Tobet, Zahniser & Baum, 1986), gerbils (Commins & Yahr, 1984) and in man (Swaab & Fliers, 1985; Swaab & Hofman, 1988). Also in birds (Japanese quail) a sexually dimorphic nucleus seems to be present in the medial preoptic area (Viglietti-Panzica *et al.* 1986).

The human SDN-POA, which corresponds to the intermediate nucleus as described by Braak & Braak (1987), can already be distinguished in the fetal brain around midpregnancy (Swaab & Hofman, 1988). In that developmental study it was found that it is only after the age of about four years that the human SDN-POA

differentiates according to sex, due to a decrease in SDN-POA cell number in women. Whereas numerous studies have implicated the medial preoptic area in the mediation and regulation of masculine sexual behaviour and endocrinological reproductive functions (Lisk, 1968; Christensen, Nance & Gorski, 1977; Silverman, Krey & Zimmerman, 1979; Döhler *et al.* 1986; Byne & Bleier, 1987), it is not possible at this time to specify the precise role of the SDN-POA within this area. Considering its striking sexual dimorphism and its androgenic sensitivity during development, the SDN-POA might be part of the neural circuitry underlying masculine reproductive processes (Anderson *et al.* 1986; Turkenburg *et al.* 1988).

The present investigation gives a quantitative description of the morphology of the human SDN-POA in normal adult subjects and investigates the existence of sexual differences. Since endocrine and reproductive functions show sex- and age-related differences (e.g. Riegle & Miller, 1981), morphometric variables of the SDN-POA are studied relative to each other, as well as to age, by using multivariate regression. To evaluate whether the sex differences and age-related changes in the morphology of the SDN-POA are specific to this region, the size, shape and cellular morphology of two other hypothalamic areas are considered as well: the suprachiasmatic (SCN) and paraventricular nuclei (PVN) (Swaab, Fliers & Partiman, 1985; Hofman, Fliers, Goudsmit & Swaab, 1988). It proved to be possible to detect internuclear disparities in the structural organisation of these hypothalamic regions.

MATERIAL AND METHODS

Data selection

Brains from 30 human subjects (13 males and 17 females), ranging in age from 10 to 93 years, were obtained at autopsy. The subjects had not been diagnosed, either clinically or neuropathologically, as having suffered from disease of the nervous system. The time interval between death and autopsy (postmortem delay) amounted to an average of 18 ± 2.1 (mean \pm S.E.M.) hours. After removal the brains were weighed and fixed in 4% formaldehyde at room temperature. After fixation for at least one month, the hypothalamic area was dissected out, dehydrated, embedded in paraffin, and cut serially in 6 μm frontal sections on a Leitz microtome. Every 25th section was mounted on a chrome-aluminium sulphate-coated slide, deparaffinised, hydrated, brought to phosphate-buffered saline and stained with thionine (for details see Swaab & Fliers, 1985; Swaab *et al.* 1985).

For the analysis of age-related changes in SDN-POA cell number, and the computation of the structural relation between SDN-POA volume and brain weight, the original data set was extended with material derived from a recent study by Swaab & Hofman (1988) on the growth and differentiation of the human SDN-POA. The measurements on the human material were performed in the same subjects as were previously used in a morphometric analysis of the SCN and PVN (Hofman *et al.* 1988), allowing us to compare the structure of these hypothalamic areas in relation to each other. Volumetric data on the rat hypothalamus were used for interspecies comparisons. These data, which are based on paraffin-sectioned brain material ($\leq 12 \mu\text{m}$), were compiled from Van den Pol (1980) and Güldner (1983) for the SCN, from Hsü *et al.* (1980) for the SDN-POA and from Hsü & Peng (1978) and Peng & Hsü (1982) for the PVN.

Morphometry

The length of the SDN-POA in the rostrocaudal direction was determined by staining every 25th section, from the lamina terminalis to the mammillary bodies with thionine. The rostral and caudal borders of the SDN-POA were assessed by subsequent staining of every 10th section in its most rostral and caudal ends. Area measurements of the cross-sectional SDN-POA were performed unilaterally by means of a Calcomp 2000 digitiser connected to a VAX 11/780 computer using a Zeiss microscope with a $\times 10$ (PLAN) objective and $\times 12.5$ (PLAN) oculars. Cell counts and measurements of cell nuclei were performed using a $\times 40$ (PLAN) objective. The SDN-POA was measured on one side of the brain.

The volume of the SDN-POA was determined by integrating the individual area measurements from the most rostral to the most caudal sections, taking into account section thickness and the magnification at which the original tracings were made (see, for example, Van Eden, Uylings & Van Pelt, 1983). The number of sections per subject in which these areas were measured was 11 ± 3 (mean \pm S.E.M.). Although volumetric analysis can be affected by differential shrinkage of the tissue (during fixation, embedding and staining), as far as we know no case of sex-related, differential shrinkage of brain tissue has ever been reported (Leibnitz, 1971; Uylings, Van Eden & Verwer, 1984). The numerical cell density of the SDN-POA was estimated by counting the total number of nuclear profiles per unit area in thionine-stained material using a discrete 'unfolding' procedure (Weibel, 1979). The procedure contained a modification proposed by Cruz-Orive (1978) along with a correction for the section thickness. For this purpose, the section with the largest cross-sectional area was selected, in which the nuclear profiles of 100–150 cells were counted per subject. The total cell number in the SDN-POA was obtained by multiplying its volume by the numerical density. Throughout this study values are expressed as mean \pm S.E.M. unless otherwise indicated.

Statistics

Morphological differences between the sexes were analysed using parametric tests (Student's *t* test, two-tailed). In addition, non-parametric tests (Wilcoxon/Mann-Whitney's U test, two-tailed) were used in order to avoid possible discrepancies derived by deviations from normality or from variance homogeneity. To evaluate the degree of sexual dimorphism a dimorphism index (DI), being defined as $100 (\bar{X}_m - \bar{X}_f) / \{(\bar{X}_m + \bar{X}_f) / 2\}$, where \bar{X}_m and \bar{X}_f are mean values from males and females, respectively. It should be noted that the dimorphism index is a less accurate measure of between-sex differences than the *t* statistic since it does not consider within-sex variance. In addition to these statistics the correlation-regression approach was applied in order to detect allometric components, which might influence the degree of dimorphism (cf. Atchley, 1978; Uylings, Hofman & Matthijssen, 1987). To test whether two samples differed significantly from each other with respect to hour and month of death the Mardia-Watson-Wheeler test was applied (see Batschelet, 1981). Throughout this study the critical level for statistical significance was taken to be 5%.

Bivariate linear models were used to describe the statistical relationships between morphological variables, in which the strength of the relationship is reflected by the product-moment correlation coefficient *r*. The regression coefficients were estimated by the standard major axis method (Hofman, Laan & Uylings, 1986; Hofman, 1988). Symmetrical tables of correlation coefficients or correlation matrices were used to

indicate the strength of relationships between variables (Sokal & Rohlf, 1981). Since the probability of making at least one Type-1 error increases with the number of tests performed, a multiple-correlation test procedure (Multistage Bonferroni test, two-tailed) was used to control for unacceptable levels of this type of error (see Larzelere & Mulaik, 1977). Results for which the null hypothesis would be rejected according to the conventional procedure are regarded as borderline. The difference in sexual dimorphism among groups was determined by comparing the correlation coefficients of both groups relative to the line of symmetry (see Hofman *et al.* 1988). The product of the residual variances of a point from the line in the x and y direction is known as the residual deviate product (RD), and is considered to be a measure of the covariance in Model II regression (Uylings, Van Eden & Hofman, 1986). In the present study the mean RD value of the sample ($\overline{RD} = \sum |RD_i|/n_i$) was used to measure the heterogeneity of residuals among groups. In the case of multiple correlation tests, low values of RD indicate that groups within a sample have similar bivariate correlation patterns. Means of RD were compared using analysis of variance. To detect non-linear patterns in SDN-POA cell number with ageing, and to reduce the effect of random error, data points were smoothed using polynomial regression and a scatterplot smoothing procedure with an equally weighted moving average ($\Delta h = 20$; $0.5 < f < 0.7$) (cf. Cleveland, 1985).

RESULTS

Size and shape of the sexually dimorphic nucleus

The human SDN-POA is an ovoid, densely packed collection of cells, characterised by its more intense staining and larger bodies as compared with the surrounding preoptic area. The SDN-POA is located in the medial part of the preoptic area, between the dorso-lateral supraoptic nucleus and the rostral pole of the paraventricular nucleus (Fig. 1; see also Swaab & Fliers, 1985). It was generally present in the same sections that contained the suprachiasmatic nucleus. The morphometric data from 13 males (aged 27–85; 51 ± 6 years) and 17 females (aged 10–93; 58 ± 7 years) were pooled according to sex. Before comparing the morphology of the SDN-POA among the sexes, factors that might influence the measurements, such as differences in brain size, age, postmortem delay, fixation time and hour and month of death were determined for both groups (cf. Zilles, 1972; Haug *et al.* 1984; Hofman, 1984). No statistically significant group differences were found for any of these parameters with the exception of the volume of the brain, which showed a marked sexual dimorphism (1400 ± 24.4 g for males and 1226 ± 35.3 g for females; $t = 3.80$, D.F. = 28, $P < 0.001$).

The rostrocaudal axis of the SDN-POA appeared to be somewhat longer in males than in females (0.823 ± 0.105 vs. 0.645 ± 0.089 mm), but the difference failed to reach statistical significance ($t = 1.37$, D.F. = 28). The maximum cross-sectional area in the central part of the SDN-POA was significantly larger in males than in females (0.446 ± 0.066 mm² and 0.220 ± 0.025 mm², respectively; $t = 3.54$, D.F. = 28, $P < 0.01$). In general the shape of the SDN-POA appeared to be elongated in females and more spherical in males. In rats a similar, but opposite, sex difference in the shape of the SDN-POA has been reported (Robinson *et al.* 1986), the nucleus being more elongated in males than in females.

The volume of the SDN-POA in adult human subjects shows a striking sexual dimorphism: the mean volume of this nucleus in males was 2.2 times larger than that in females (Table 1). The total number of cells in the male SDN-POA was 2.1 times larger than in the female. These results are in accordance with previous findings by

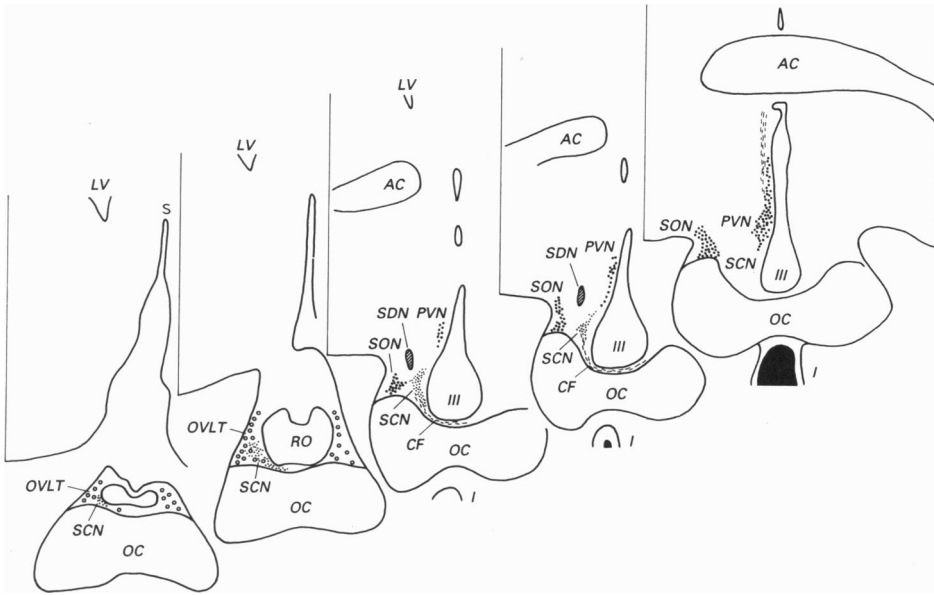


Fig. 1. Topography of the sexually dimorphic nucleus (SDN) in the preoptic area of the human hypothalamus. Third ventricle, III; anterior commissure, AC; infundibulum, I; lateral ventricle, LV; optic chiasma, OC; organum vasculosum of the lamina terminalis, OVLT; recessus opticus, RO; septum, S; suprachiasmatic nucleus, SCN; paraventricular nucleus, PVN; supraoptic nucleus, SON; commissural fibres of the suprachiasmatic nucleus, CF. (From Swaab & Fliers, 1985; copyright 1985 by the AAAS.)

Swaab & Fliers (1985). To investigate whether the sexual dimorphism in the volume of the SDN-POA is correlated with the dimorphism in brain size between the sexes, the allometric relations between both variables were determined. For adult males (brain weight: 1100–1700 g) the relationship was given by

$$\log V = 4.80 \log E - 15.842$$

($\alpha = 4.80 \pm 0.87$; $r = 0.453$; D.F. = 24; $P < 0.05$), where V = volume of the SDN-POA (mm^3) and E = brain weight (g) (Fig. 2). A similar allometric relationship could be derived for adult females (brain weight: 1000–1500 g), yielding

$$\log V = 6.77 \log E - 22.104$$

($\alpha = 6.77 \pm 1.05$; $r = 0.646$; D.F. = 24; $P < 0.001$). The slopes of the standard major axes were homogeneous ($t = 1.47$, D.F. = 13.8, $P > 0.15$), indicating that the SDN-POA volume scales in a similar, *non-linear*, way to brain weight in adult males and females (Fig. 2). Furthermore, analysis of covariance comparing the residual variances among and within groups, when controlled for the sex differences in brain size, revealed that after allometric size correction, there are no significant differences between the sexes for the scaled or adjusted trait ($F[2, 101] = 0.193$). Thus, we may conclude that the sex differences in the volume of the human SDN-POA in adulthood can be explained, to a great extent, by the sexual dimorphism in general brain size. It is important to note that there were no statistically significant sex differences in either cell density or size of the cell nuclei (Table 1).

Bivariate linear models were then used to describe the statistical relations between

Table 1. Morphometry of the human sexually dimorphic nucleus

Variable ¹	Males (n = 13)	Females (n = 17)	Dimorphism index (%) ²	Statistics ³	
				t _s	U _s
Volume (mm ³)	0.182 ± 0.0222	0.084 ± 0.0116	73.7	4.178***	188***
Cell density (× 10 ³ mm ⁻³)	159.4 ± 12.05	151.9 ± 8.06	4.82	0.536	114.5
Total cell number (× 10 ³)	27.56 ± 3.172	13.20 ± 2.152	70.5	3.877**	186**
Cell-nuclear diameter (μm)	6.36 ± 0.149	6.49 ± 0.136	-2.02	-0.641	127
Brain weight (g)	1400 ± 24.4	1226 ± 35.3	13.3	3.802***	185.5**

¹ Values are given as mean ± S.E.M.

² Dimorphism index = $100(\bar{X}_m - \bar{X}_f)/((\bar{X}_m + \bar{X}_f)/2)$; see Material and Methods.

³ t_s, Student's t test; U_s, Wilcoxon/Mann-Whitney U test.

Note: *** P ≤ 0.001; ** 0.001 < P ≤ 0.01; * 0.01 < P ≤ 0.05.

Table 2. Correlation matrix of bivariate linear regression of age, brain weight and four morphometric parameters of the human sexually dimorphic nucleus (SDN-POA)^a

	1	2	3	4	5	6
	Males (n = 13)					
(1) Age	—					
(2) Brain weight	-0.368	—				
(3) SDN-POA volume	-0.638 ^b	0.199	—			
(4) SDN-POA cell density	0.106	-0.373	-0.497	—		
(5) SDN-POA total cell number	-0.707*	0.097	0.846***	0.023	—	
(6) Cell-nuclear diameter	0.171	0.519	0.200	-0.492	-0.042	—
	Females (n = 17)					
(1) Age	—					
(2) Brain weight	-0.721**	—				
(3) SDN-POA volume	-0.753***	0.468	—			
(4) SDN-POA cell density	-0.311	-0.048	0.298	—		
(5) SDN-POA total cell number	-0.738***	0.379	0.962***	0.528 ^b	—	
(6) Cell-nuclear diameter	0.194	-0.180	0.129	-0.516 ^b	-0.015	—

^a A two-tailed multi-stage Bonferroni procedure was used to obtain the data; see Material and Methods.

^b Borderline significance; P_c < 0.05.

Note: *** P ≤ 0.001; ** 0.001 < P ≤ 0.01; * 0.01 < P ≤ 0.05.

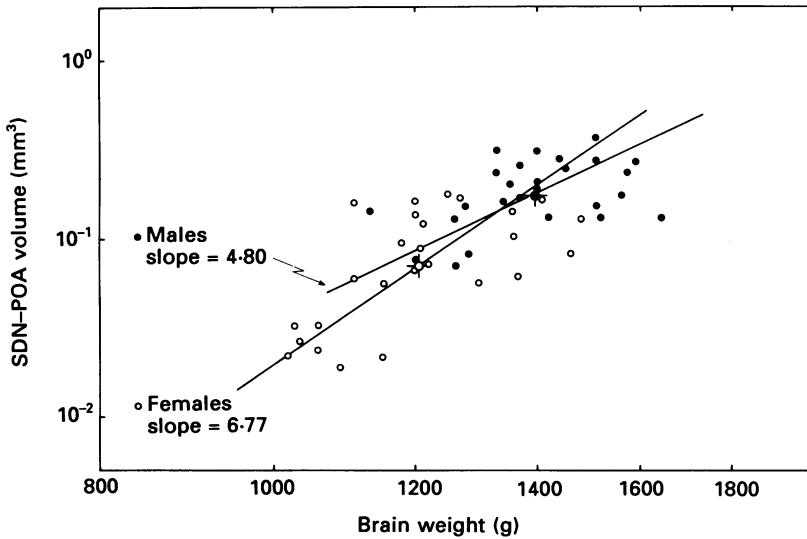


Fig. 2. The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) as a function of brain weight in human adults. Log-log scale. Note that the slopes of the regression lines are homogeneous ($P > 0.15$), indicating that the SDN-POA volume scales in a similar way to brain weight in adult males (●) and females (○). Equations are given in the text.

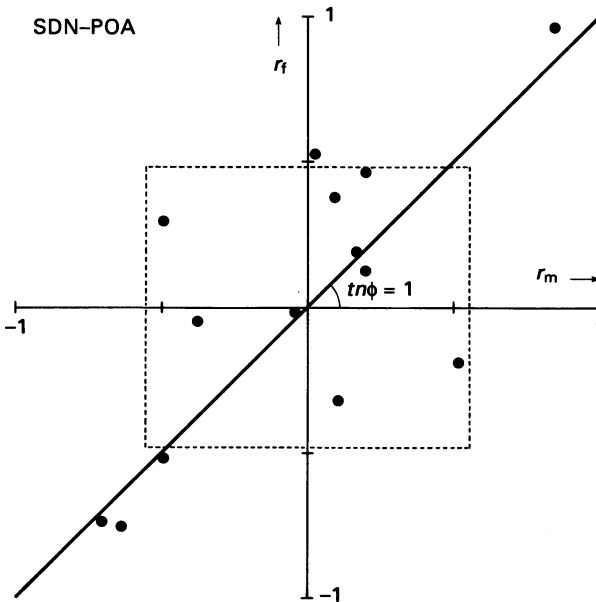


Fig. 3. Correlation diagram for the human sexually dimorphic nucleus of the preoptic area (SDN-POA), relating the correlation coefficients of males (r_m) and females (r_f). Values were obtained from bivariate morphometric regression analysis (Table 2). Sexual disparity was determined by computing the residual variance of points from the line of symmetry ($r_f = r_m$) (see Material and Methods). The rectangle demarcated by the interrupted lines represents the area within which both correlation coefficients are not statistically significant ($\alpha = 0.05$).

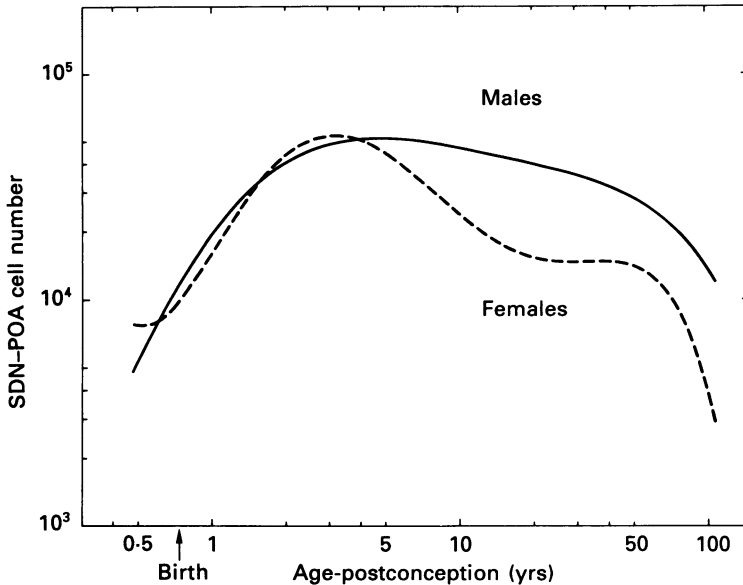


Fig. 4. Development and sexual differentiation of the human sexually dimorphic nucleus of the preoptic area (SDN-POA). Cell numbers reach a peak value around 2–4 years postnatally, after which a sexual differentiation occurs due to a reduction in cell number in the SDN-POA in females, whereas the cell number in males remains approximately unchanged up to the age of 50 years. The curves are fifth degree polynomial functions fitted to the data compiled from Swaab and Hofman (1988), with $F_5(5, 49) = 10.05$, $P < 0.001$ for males and $F_5(5, 39) = 7.32$, $P < 0.001$ for females.

the morphological variables in question. These bivariate comparisons, like the findings for the absolute measurements, showed a noticeable sexual dimorphism (Table 2). Among the most remarkable findings were the sex-dependent effect of age on the volume of the SDN-POA and the sexual discrepancy in the relationship between the number of cells and the cell density. Thus in females an increase in cell number was accompanied by an increase in cell packing, whereas in males these variables were not correlated. To get an impression of the sexual differences of the complete set of relationships, the correlation coefficients of females were plotted against those of males in a correlation diagram (Fig. 3). The mean residual deviate product (RD; see Material and Methods), which in the present study is a measure of (sexual) heterogeneity, yielded a value of 0.132 ± 0.053 , which is significantly different from zero ($t = 2.47$, D.F. = 13, $P < 0.05$). These findings indicate that there is a sexual dimorphism in the way the variables in question are interrelated, and suggest that there are sexual differences in the internal structural organisation of the human SDN-POA.

SDN-POA dimorphism and ageing

By analogy with observations in many mammalian species, the human brain is believed to undergo sexual differentiation during development due to an organising effect of gonadal hormones (see Swaab & Hofman, 1984). Until recently, however, no information was available with regard to the development of sexually dimorphic characteristics in the human hypothalamus. The description of the SDN-POA in man (Swaab & Fliers, 1985) has made it possible to determine at what age a specific hypothalamic structure begins to show sexual differentiation and how these

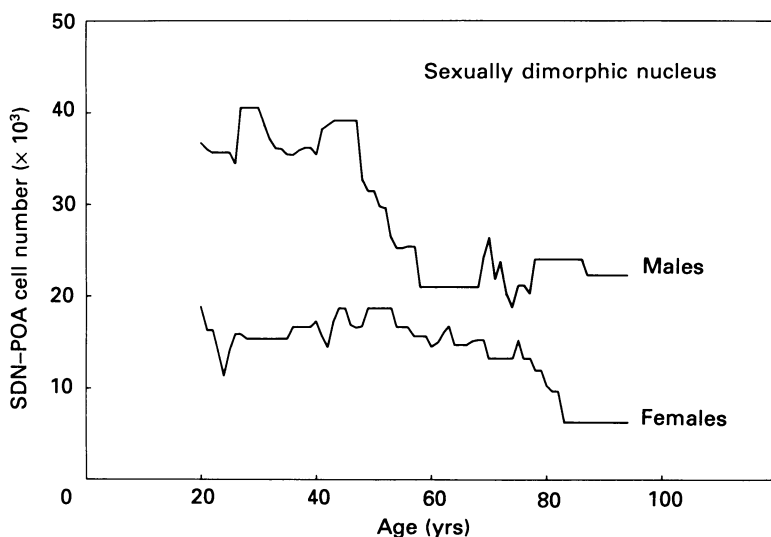


Fig. 5. Age-related changes in the total cell number of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in the human hypothalamus. The general trend in the data is enhanced by using smoothed growth curves (see Material and Methods). Note that in males SDN-POA cell number steeply declines 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.

morphological differences between the sexes are related to the age of the subjects. Swaab & Hofman (1988) have demonstrated that the volume and cell number of the human SDN-POA reach a peak value between the age of 2 and 4 years postnatally, after which sexual differentiation becomes manifest due to a decrease in cell number in females. As can be seen in polynomial growth curves of the human SDN-POA (Fig. 4), the second half of the first decade of postnatal life is a critical period in the development and maturation of the sexual dimorphism. In rats, sexual differentiation of the volume of the SDN-POA has been reported to occur during the first ten days postnatally (Hsü *et al.* 1980; Jacobson *et al.* 1980).

When we consider the development of the SDN-POA in adulthood, using a scatterplot smoothing procedure, we see that the SDN-POA cell number in males remains approximately unchanged up to the age of 50 years, after which it declines steeply (Fig. 5). In the period from 45 to 60 years we found the cell number to diminish at a rate of 3% per year. After the age of 60 years, the cell number in the male SDN-POA does not decrease any further. In females, the cell number starts to decrease for the second time after the age of 50 years. After a period of relative stability, from 70 years onwards, a third, and more dramatic reduction in SDN-POA cell number in females could be detected. In the period between 75 and 85 years, the cell number in the SDN-POA of females decreases at a rate of 4.8% per year, leading to values which are only 10–15% of the peak value found at 2–4 years postnatally. These results clearly demonstrate that the sex differences in total cell number within the human SDN-POA are by no means constant throughout adult life. As can be seen in Figure 6, the largest discrepancies in SDN-POA cell number between the sexes are found around the age of 30 years and in people older than 80 years, whereas the sexual dimorphism is least around the age of 60 years.

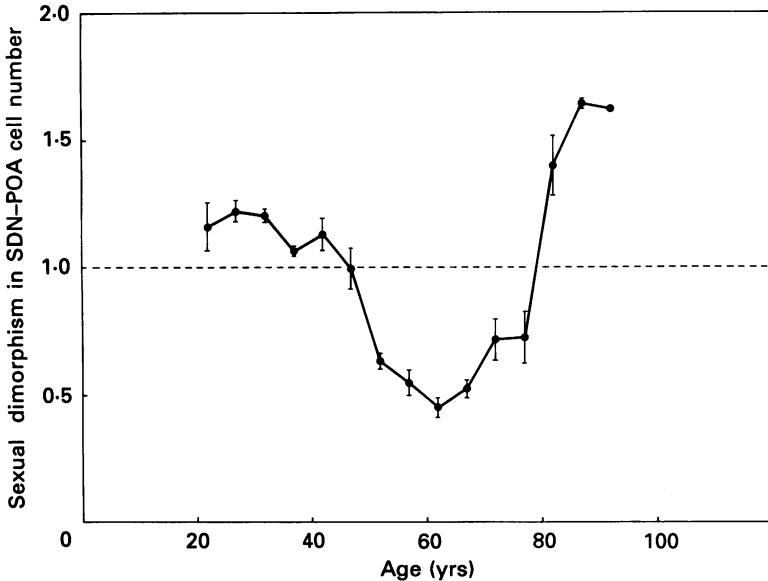


Fig. 6. Sexual dimorphism in SDN-POA cell number in human adults as a function of age. Values are means \pm S.E.M. and based on normalised five-year averages of smoothed data (cf. Fig. 5). The dashed line is the normalised mean dimorphism for adults. Notice that the sexual differentiation in SDN-POA cell number is not constant throughout adult life, but varies with age.

The volume of the SDN-POA was found to decrease in senescence in a similar way as did the cell number. Comparing the volume of SDN-POA in young adults (20–30 years) with that in aged people (70–90 years) we found a volume reduction of 43% in males and 62% in females, whereas the diminution of the total brain amounted to only 6%. The size of the other subcortical regions in the human brain, such as the thalamus, striatum and basal nuclei have also been reported to diminish in old age (Haug *et al.* 1983), but with a volume reduction of only 20–25%, these parts do not show the dramatic level of degeneration found in the SDN-POA. These differential rates of volume reduction confirm previous findings indicating that each part of the human brain has its own history of ageing (Eggers, Fischer & Haug, 1981; Haug *et al.* 1984), but, in addition, the amount of cell loss within a region during a lifetime seems to depend upon the sex of the subject.

Internuclear comparisons

Comparing the overall morphology of the SDN-POA with that of two other cell groups in the preoptic area of the human hypothalamus – the suprachiasmatic nucleus (SCN) and paraventricular nucleus (PVN) (cf. Swaab *et al.* 1985; Hofman *et al.* 1988), the size of the SDN-POA in males was found to be similar to that of the SCN (Table 3). In females, on the other hand, the SCN was 2.8 times larger than the SDN-POA and contained 3.5 times as many cells. The morphological differences between the SDN-POA and SCN, however, were minor when compared with the dimensions of the PVN. As can be seen in Figure 7, the PVN is a hypothalamic area which is considerably larger and contains far more cells than either the SDN-POA or the SCN (see Hofman *et al.* 1988). The closer the relationship between cell number and volume, indicated by the correlation coefficient r in Figure 7, the smaller the variance of cell density within a region, since $(N) \equiv (V)(N_v)$, where N = total cell number, V =

Table 3. Morphometric differences between the suprachiasmatic nucleus (SCN) and sexually dimorphic nucleus (SDN-POA) in the human brain

Variable ¹	Suprachiasmatic nucleus	Sexually dimorphic nucleus	Dimorphism ² index (%)	Statistics ³	
				t _s	U _s
		Males (n = 13)			
Volume (mm ³)	0.239 ± 0.0175	0.182 ± 0.0222	27.1	2.015	116
Cell density (× 10 ³ mm ⁻³)	188.4 ± 15.69	159.4 ± 12.05	16.7	1.466	109
Total cell number (× 10 ³)	45.23 ± 4.753	27.56 ± 3.172	48.6	3.092**	135**
Cell-nuclear diameter (µm)	5.39 ± 0.121	6.36 ± 0.149	-16.5	-5.054***	159.5***
		Females (n = 17)			
Volume (mm ³)	0.239 ± 0.0256	0.084 ± 0.0116	95.9	5.515***	268.5***
Cell density (× 10 ³ mm ⁻³)	199.7 ± 14.38	151.9 ± 8.06	27.2	2.709*	222**
Total cell number (× 10 ³)	45.68 ± 4.188	13.20 ± 2.152	110.3	6.898***	274***
Cell-nuclear diameter (µm)	5.57 ± 0.155	6.49 ± 0.136	-15.3	-4.462***	251.5***

¹ Values are given as mean ± S.E.M.² Dimorphism index = $100 (\bar{X}_m - \bar{X}_f) / ((\bar{X}_m + \bar{X}_f) / 2)$; see Material and Methods.³ t_s, Student's *t* test; U_s, Wilcoxon/Mann-Whitney U test.Note: *** $P \leq 0.001$; ** $0.001 < P \leq 0.01$; * $0.01 < P \leq 0.05$.

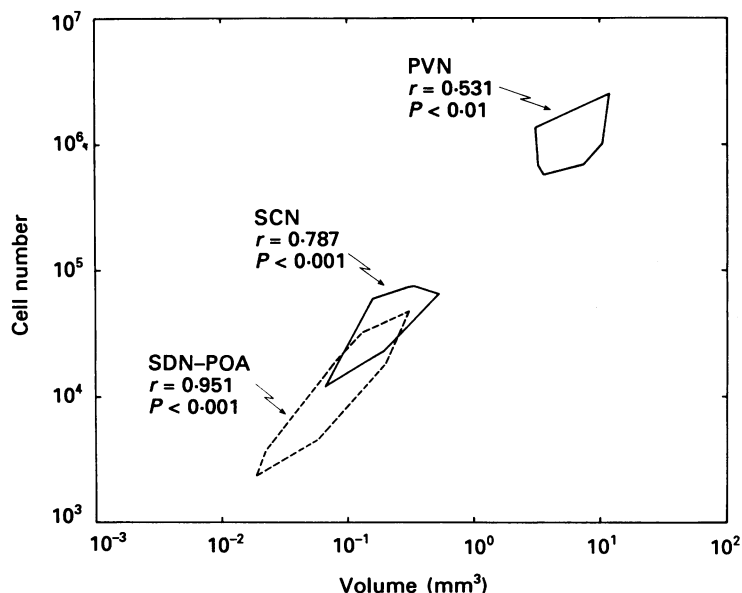


Fig. 7. The total number of cells in hypothalamic regions in the human brain as a function of their volume. Log-log scale. Minimum convex polygons, containing all bivariate data of both sexes ($n = 30$), were drawn for the following hypothalamic areas: the sexually dimorphic nucleus of the preoptic area (SDN-POA), the suprachiasmatic nucleus (SCN) and the paraventricular nucleus (PVN). Correlation coefficients are indicated by r . The closer the relationship between cell number and volume, the smaller the variance of cell density within a region. Neither of the slopes of the standard major axes differed from one.

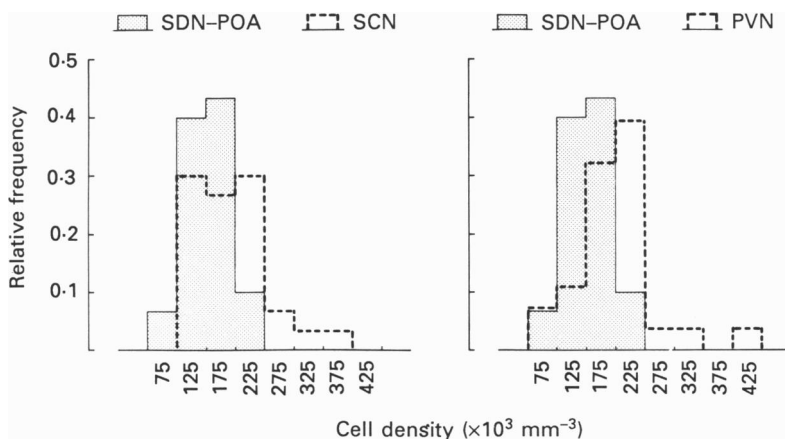


Fig. 8. Probability functions of cell density for the human sexually dimorphic nucleus (SDN-POA), suprachiasmatic nucleus (SCN) and paraventricular nucleus (PVN). Note that the distribution of the cell density sample of the SDN-POA does not differ significantly from that of other hypothalamic areas.

volume and N_v = numerical cell density. Accordingly, the coefficients of variance of the mean cell densities of the areas were 0.241, 0.295 and 0.331 for the SDN-POA, SCN and PVN respectively, indicating that the variance in SDN-POA cell size between subjects is less than in either the SCN or PVN.

Analysis of the cellular morphology of these hypothalamic regions revealed that the frequency distributions of their cell densities did not differ significantly (Fig. 8). On the other hand, probability functions of the mean diameters of the cell nuclei showed that

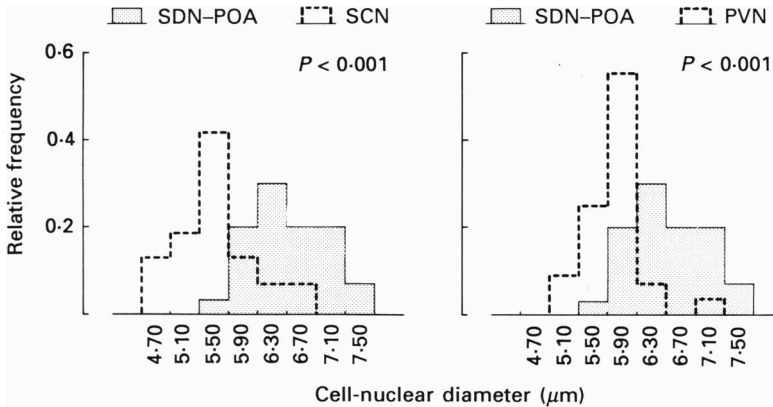


Fig. 9. Probability functions of the mean diameter of the cell nucleus for the human sexually dimorphic nucleus (SDN-POA), suprachiasmatic nucleus (SCN) and paraventricular nucleus (PVN). Note the difference in cell-nuclear diameter between the hypothalamic regions (Kolmogorov-Smirnov test), indicating that the human SDN-POA contains relatively large cells.

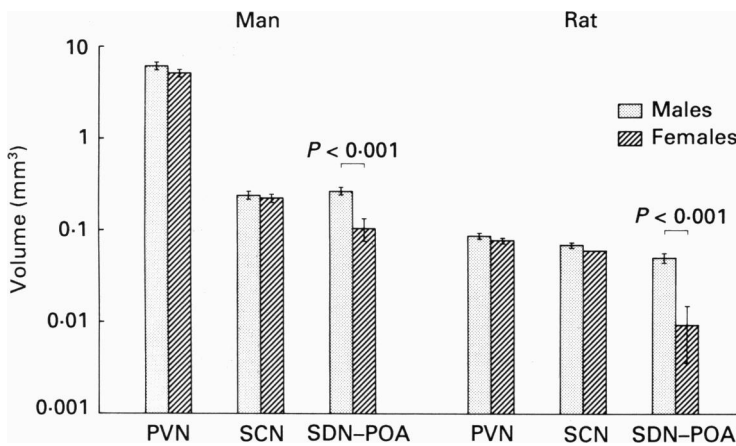


Fig. 10. Volumes of hypothalamic regions in young adult rats (2-5.5 months) and in man (20-40 years). Values represent the mean volume (\pm S.E.M.) from one hemisphere. The variance of the SCN volume in female rats could not be determined. For the sources of data see Material and Methods. The sexual differences in the volume of the SDN-POA were statistically significant, both in man and in the rat. Abbreviations are the same as in Fig. 9.

the cell sample of the SDN-POA was derived from a population with a size distribution different from that of the SCN or PVN ($D = 0.700$, $P < 0.001$ for SDN-POA vs. SCN, and $D = 0.660$, $P < 0.001$ for SDN-POA vs. PVN, see Fig. 9). These differences in both shape and location of the size distributions of the cell nuclei indicate that the human SDN-POA contains a higher percentage of large cells (mean nuclear diameter of cells ($\phi = 6.43 \pm 0.100 \mu\text{m}$) than either the SCN ($\phi = 5.49 \pm 0.078 \mu\text{m}$) or the PVN ($\phi = 5.79 \pm 0.102 \mu\text{m}$).

To compare the size of the human SDN-POA, SCN and PVN and their sexual dimorphism with that of other species, we used the volumetric data derived from rat studies (Fig. 10). It appears that in the rat the SCN and PVN are almost equal in size, whereas in human brains the PVN is considerably larger than the SCN. This species-specific difference may be due to the relatively small size of the human SCN, which is

only 3.7 times larger than the SCN of the rat whereas the entire human hypothalamus is approximately 70-fold larger than that of the rat (cf. Hofman *et al.* 1988). The PVN volume, on the other hand, accounts for an equal fraction of about 0.3% of the hypothalamic volume in both species.

The volume of the human SDN-POA in young adults (20–40 years), like that of the SCN, is relatively small (Fig. 10) and accounts for only 0.01% of the hypothalamic volume in males and 0.006% in females, whereas in young adult rats (2–5.5 months) the corresponding fractions are 0.19% and 0.04%, respectively. Whether this relative reduction in the size of the human SDN-POA has any functional significance or is merely an allometric scaling phenomenon is not known (see Discussion). The sexual dimorphism in the volume of the SDN-POA, being absent in the SCN and PVN of both man and rat, seems to be more prominent in rats ($\delta/\text{♀} \approx 5$) than in the human brain ($\delta/\text{♀} \approx 2$). In guinea-pigs the sexual differentiation of the SDN-POA has been reported to be less pronounced than in rats, being approximately 4 times larger in males than in females (Hines *et al.* 1985). Whether these species-specific sexual differences in the morphology of the SDN-POA have a functional equivalent, for example, in the way mammals differ in their regulation of sexually differentiated behavioural functions, remains to be shown.

DISCUSSION

The present study has established that there is a striking sexual dimorphism in the size, shape and cellular morphology of the SDN-POA in the human hypothalamus. These results confirm the sexual differences in the SDN-POA previously described by Swaab & Fliers (1985). The sexual dimorphism of the SDN-POA, caused by a dramatic cell loss in females during early childhood (Swaab & Hofman, 1988), seems to be a phenomenon that is specific among hypothalamic nuclei to the SDN-POA. The suprachiasmatic nucleus (SCN), supraoptic nucleus (SON) and paraventricular nucleus (PVN) failed to show sexual differences in the same subjects (Swaab *et al.* 1985; Hofman *et al.* 1988; Goudsmit, Hofman, Fliers & Swaab, unpublished data). These results clearly demonstrate that every part of the hypothalamus has its own characteristic degree of sexual dimorphism, which depends, in addition, upon the species considered. In rodents, for example, the sexual dimorphism of the SDN-POA has been found to be more marked than in man. Whether such differences in sexual differentiation of the SDN-POA among mammals have any functional significance is not known. There is also a possibility, however, that this species-specific dimorphism is simply a scaling effect, in which the relationship between brain size and SDN-POA volume depends upon the genotypic sex. If true, the sexual dimorphism of the SDN-POA in small mammals, such as rodents and insectivores, would be larger than in more encephalised species, such as primates.

The present investigation describes, for the first time, the relationship between the volume of the SDN-POA and brain weight within a species. The analyses indicate that the volume of the human SDN-POA is significantly related to brain weight in both males and females, suggesting a size dependency which might explain the sexual dimorphism of the SDN-POA. It should be noted that these non-linear, allometric relations between SDN-POA volume and brain weight only hold for adult brains; the development of the SDN-POA in the pre-adult phase (as measured by the volume and cell number) is itself sexually dimorphic and seems to be independent of changes in brain size. Jacobson *et al.* (1980) arrived at a similar conclusion for the ontogeny of the SDN-POA in rats. Jacobson's findings, on the other hand, are based on ratios, in

which the volume of the area was expressed relative to linear parameters, such as the height of the brain or POA, instead of on allometric correction methods. Ratio adjustments, however, exhibit undesirable and unpredictable properties (Atchley, 1978; Uylings *et al.* 1987) and should be avoided whenever possible. Instead log residuals in bivariate space should be used to arrive at size-adjusted variables (Hartman, 1983; Hofman, 1988).

According to Haug and his co-workers (1983), every part of the human brain has its own history of ageing. Our data extend this finding by demonstrating that every sexually dimorphic structure in the human brain has its own sex-dependent pattern of growth and decay (cf. Swaab & Hofman, 1984, 1988; Kraftsik, Clarke, Innocenti & Van der Loos, 1987). The SDN-POA cell number in females has been reported to decline from the age of 4 years (Swaab & Hofman, 1988). From this age onwards cells disappear in two or three phases, dropping to values which are only 10–15% of the cell number found at 2 years postnatally, being smaller than the values found at birth. These periods of cell death are interrupted by periods in which little or no discernible cell loss can be detected. A similar temporal pattern of neural degeneration in early childhood has been found in human micrencephalics (Hofman, 1984). The brains of these mentally retarded patients are as a rule entirely reduced in size, with only the cerebellum sometimes being disproportionately large. Brain growth in micrencephalics, although impaired, continues steadily up to the age of 3–5 years, after which it starts to decline. Thus, micrencephalic patients have a significantly lower brain weight in adolescence than in early childhood.

In males, a major reduction in SDN-POA cell number was found between the age of 50–60 years. In addition to changes in the hypothalamus, increasing age is accompanied by decreased sexual activity and deterioration of gonadal function (for reviews see Riegle & Miller, 1981; Sonntag, 1987). A substantial decrease in free testosterone concentrations, for example, has been observed in healthy human males between 45–65 years, as well as a reduced ovarian oestradiol and progesterone secretion in postmenopausal females (Vermeulen, 1976; Albeaux-Fernet, Bohler & Karpas, 1978; Deslypere & Vermeulen, 1984). Whether the reduction in SDN-POA cell number with ageing is a direct consequence of these age-related alterations in gonadal function, or vice versa, is not clear. A time-course relationship between ageing effects on the cellular morphology of the SDN-POA and age-related deterioration of reproductive functions might give a clue as to the putative role of the SDN-POA in the neural circuitry underlying sexual behaviour and reproductive processes.

Except for the results presented here, no reports are available in which the morphology and sexual dimorphism of the human SDN-POA is compared with those of other regions of the preoptic/anterior hypothalamus. Thus, the PVN in the human hypothalamus was found to be considerably larger than the SCN or SDN-POA, the latter nuclei being similar in size. In rats, on the other hand, the volumes of the SDN-POA, SCN and PVN are approximately the same, except for the female SDN-POA, which is smaller. This species discrepancy might be a scaling effect and does not necessarily have to be a consequence of an increased functional activity of the human PVN or a diminished significance of the SCN or SDN-POA in man. In fact, the relatively small number of neurons in the SCN and SDN-POA may be sufficient adequately to generate, or to modulate, physiological and endocrinological processes in small and large mammals alike, independently of the morphology and intrinsic organisation of related hypothalamic cell groups.

SUMMARY

The sexually dimorphic nucleus of the preoptic area (SDN-POA) in the human hypothalamus is an ovoid, densely packed collection of large cells. The size, shape and cellular morphology of the SDN-POA was examined in relation to sex and age in adult human subjects. In this region the following parameters were measured: length of the rostrocaudal axis, maximum cross-sectional area, volume, numerical cell density, total number of cells, and the diameter of the cell nucleus.

The SDN-POA was elongated in females and more spherical in males. The mean volume and total cell number were markedly sexually dimorphic: the volume of the SDN-POA was 2.2 times as large in males as in females and contained 2.1 times as many cells. No sex differences were observed in either cell density or mean diameter of the cell nuclei. Furthermore, multivariate regression analysis revealed that there are also sex-linked differences in the structural organisation of the human SDN-POA, finding expression in the way the morphometric parameters are interrelated.

Of the parameters measured, only the volume and cell number of the SDN-POA showed a dramatic decrease with ageing. The reduction in cell number, however, was not constant throughout adulthood but was found to depend upon sex and age. In males, a major reduction in SDN-POA cell number was observed between the age of 50–60 years. In females, cell death was found to be more prominent than in males, especially among old people ($t > 70$ years), dropping to values which were only 10–15% of the cell number found in early childhood. In conclusion, the human SDN-POA has a sex-dependent pattern of ageing.

Finally, the morphology of the SDN-POA was compared with that of other hypothalamic regions – the suprachiasmatic nucleus (SCN) and the paraventricular nucleus (PVN) – both in man and in rat. Species-specific differences in the dimensions of these nuclear regions are discussed in the light of their assumed functional significance.

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