ANNUAL VARIATIONS IN THE VASOPRESSIN NEURON POPULATION OF THE HUMAN SUPRACHIASMATIC NUCLEUS

M. A. HOFMAN*, J. S. PURBA and D. F. SWAAB

Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

Abstract—The mammalian suprachiasmatic nucleus is considered to be the major component of the biological clock, involved in the temporal organization of a wide variety of physiological and behavioral processes. The present study was conducted to investigate whether there are diurnal or annual variations in the morphology of the vasopressin-containing neuron population of the suprachiasmatic nucleus in human beings. To that end, the brains of 48 human subjects were investigated.

A marked annual variation was observed in the volume and vasopressin cell number of the human suprachiasmatic nucleus: the volume of the vasopressin cell population was, on average, 2.5 times larger in October–November than in May–June and contained 2.7 times as many vasopressin-immunoreactive neurons. In general, the annual cycle of the human suprachiasmatic nucleus showed a non-sinusoidal pattern with a maximum in early autumn, a lower plateau in winter and a deep trough in late spring and early summer. In contrast, no such seasonal variations could be detected in suprachiasmatic nucleus vasopressin numerical cell density or cell-nuclear diameter. The number of vasopressin-immunoreactive neurons in the paraventricular nucleus of the hypothalamus, on the other hand, did not show any significant periodic changes over the year, indicating the specificity of the suprachiasmatic nucleus rhythm. In contrast with the annual cycle of the suprachiasmatic nucleus, no significant diurnal variations were observed in any of these parameters.

In conclusion, the findings indicate that photoperiod may be considered a potential environmental factor controlling the activity of the vasopressin system of the human suprachiasmatic nucleus.

The human suprachiasmatic nucleus (SCN) is a collection of parvocellular neurons located in the basal part of the anterior hypothalamus. The homology of this cell group with the SCN in rodents and non-human primates has only recently been established.^{28,56,58} The SCN is generally considered to be the principal component of a biological clock, which generates and coordinates a wide spectrum of biochemical, physiological, endocrine and behavioral rhythms (for reviews, see Refs 32,50,63). A direct neural pathway from the retina to the SCN, the retinohypothalamic tract, which exists in all mammals studied so far, including man⁵¹ appears to be both necessary and sufficient for synchronization of the period and phase of circadian rhythms to the environmental light–dark cycle. Furthermore, indirect retinal projections have been found to enter the SCN via the lateral geniculate nucleus and raphe nuclei.^{18,34,37}

Consistent with its role in the temporal organization of circadian processes, investigations in rodents and non-human primates suggest that the SCN is also involved in the seasonal control of reproductive and metabolic phenomena.^{14,25,62,64,69} In fact, many of the functions that exhibit seasonal cycles in mammals, such as sexual behaviour, energy metabolism, food

intake and hibernation are regulated by this time-keeping system in the brain.^{4,17,19,50}

In recent years it has become clear that in mammals the pineal gland and the SCN are essential for the regulation of these photoperiodic responses.^{8,16,21,31,33,36,42,65,66} Interruption of the retinohypothalamic pathway, or changes in environmental lighting conditions were found to influence the rhythm of the suprachiasmatic pacemaker, and consequently the synthesizing activity of the pineal gland. In mammals, the circadian synthesis and secretion of the hormone melatonin exhibited by the pineal gland is generated by oscillators in the SCN and is communicated to the pineal via a complex multisynaptic pathway.^{5,26,35} Disruption of any portion of this pathway from the SCN to the pineal gland abolishes melatonin rhythmicity. Several studies have shown that the vasopressin system in the SCN may be involved in the transmission of photic information from the retina to the pineal gland and other parts of the brain.^{6,8,20,33,53} These findings strongly suggest that the endocrine activity of the mammalian pineal is under neural control, and receives a major input from the suprachiasmatic nucleus. In fact, environmental light impulses form a continuous chain with the SCN–pineal complex which seems to be essential for the light-mediated control of reproductive functions. This means that in addition to its role as a circadian pacemaker, the SCN may also be involved in the seasonal timing of a number of physiological and

*To whom correspondence should be addressed.

Abbreviations: PVN, paraventricular nucleus of the hypothalamus; SCN, suprachiasmatic nucleus.

behavioural processes by regulation of the photoperiod-dependent changes in melatonin secretion.^{2,8,38}

Since the environmental light-dark cycle is the main Zeitgeber for circadian and seasonal rhythms in most species, including man,^{3,17} photic information may have a synchronizing effect on the clock mechanism of the SCN by inducing changes in the activity of certain groups of neurons. Our recent findings seem to support this idea, suggesting that the vasopressin-synthetic activity of the SCN in human beings fluctuates significantly over the year.²³ The aim of the present study was to determine whether the daily light-dark cycle as well as annual variations in photoperiod affect the cytomorphology of the vasopressin system in the human SCN.

EXPERIMENTAL PROCEDURES

Subjects

Brains of 48 human subjects (24 males and 24 females), ranging in age from six to 91 years (43.4 ± 3.6 years;

mean $\pm$ S.E.M.), were obtained at autopsy (Table 1). These brains, which were from individuals who had no known neurological or psychiatric disorders, were collected and neuropathologically examined either in the Free University Hospital (Dr W. Kamphorst) or the Academic Medical Center (Dr D. Troost), both in Amsterdam, The Netherlands, located at 52° Northern latitude, during the period 1981–1989. The time of death of the subjects was uniformly distributed over the 24-h period as well as over the year (Rayleigh test; $P > 0.2$ for both periods). In nine cases the exact hour of death was not recorded.

Histology

Brains were removed from their skulls within 48 h *post mortem*, weighed and placed directly into 10% formaldehyde where they were stored at room temperature for about one month prior to histological preparation. The hypothalamic area containing the suprachiasmatic nucleus was dissected from each brain, dehydrated in graded ethanol and embedded in paraffin. Serial 6- μ m frontal sections were cut on a Leitz microtome, mounted on chrome-aluminum sulphate-coated slides, deparaffinized, hydrated, brought to phosphate-buffered saline. Volume and vasopressin cell numbers were determined in two hypothalamic nuclei, the SCN and the paraventricular nucleus (PVN).^{22,58} The PVN,

Table 1. Brain material

Patient number	Sex	Age (years)	Brain weight (g)	Hour of death*	Month of death	Clinical diagnosis
30587	m	6	1550	08.00	Oct	Intestinal necrosis
87378	f	7	1320	07.15	Nov	Pylocystic astrocytoma
87072	f	9	1550	—	May	Ischemichypoxic brain damage
81306	f	10	1270	09.00	Dec	Cardiac failure; bronchopneumonia
87191	f	13	1250	04.00	Jun	Histiocytic lymphoma; hemorrhagic diathesis
87444	m	14	1840	—	Dec	Congenital myopathia; cardiac failure
80280	f	15	1480	13.00	Dec	Cerebellar hematoma; hydrothorax
81043	m	16	1940	18.00	Feb	Multiple traumata; cerebral contusion
81241	m	19	1900	00.30	Oct	Retroperitoneal chondrosarcoma
83306	m	22	1570	18.15	Oct	Lymphatic leukemia
87110	m	23	1310	11.00	Mar	Encephalitis in brainstem
87626	m	27	1330	23.00	Feb	Coma
82181	m	27	1560	—	Jul	Drug addiction; sepsis (<i>S. aureus</i>)
81058	m	28	1510	10.00	Mar	Medial cerebral artery aneurism; lung emboli
86414	m	28	1450	—	Nov	Guillain-Barré syndrome
85124	f	29	1150	20.45	May	Alcoholic cirrhosis; hemorrhagic ascites
84186	m	29	1400	21.30	Aug	Congenital heart disease; cardiac failure
81255	f	30	1460	11.45	Nov	Intramural dissecting haematoma of coronary artery
81251	m	31	1330	20.15	Nov	Multiple traumata; small subarachnoidal hemorrhage
86354	f	33	1035	—	Oct	Adenocarcinoma; multiple metastases
80271	f	35	1200	05.00	Dec	Acute lymphoblastic leukemia
84018	f	36	1420	21.00	Jan	Multiple fractures; rupture of thoracic aorta
84248	m	37	1370	01.30	Oct	Bronchopneumonia
87260	m	37	1510	—	Jul	Alcohol intoxication, combined with benzodiazepines
81012	f	38	1360	12.15	Jan	Cervix carcinoma
87345	m	41	1440	—	Oct	Cerebral contusion; lung emboli

continued

Patient number	Sex	Age (years)	Brain weight (g)	Hour of death*	Month of death	Clinical diagnosis
81093	m	42	1510	11.15	Apr	Metastatic bronchogenic carcinoma; pneumothorax
81267	m	43	1260	12.30	Nov	Non-Hodgkin lymphoma; sepsis
83173	f	46	1360	06.15	Jun	Adrenal carcinoma; post-operative hemorrhage
87271	m	47	1620	—	Jul	Amyotrophic lateral sclerosis
81020	f	50	1210	07.00	Jan	M. Kahler; renal insufficiency
82165	f	52	1370	05.45	Jul	Metastatic mamma carcinoma
86403	f	53	1410	14.00	Nov	Chronic myelocytic leukemia; blastomatosis
82161	f	57	1220	11.45	Jul	Polymyalgia rheumatica; endocarditis; lung emboli and hemorrhagic infarction
80087	f	59	1110	—	Mar	Acute leukemia; hemorrhagic diathesis
4724	m	59	1350	06.30	Sep	Pulmonary emphysema; pneumothorax
4727	m	61	1400	11.30	Sep	Myocardial infarction; cardiac failure
84049	m	63	1420	00.45	Mar	Myocardial infarction; cardiac failure
81014	f	64	1090	02.00	Jan	Hemorrhagic peptic ulcer; hypovolemic shock; renal insufficiency
4725	f	72	1200	16.45	Sep	Endocarditis lenta; glomerulo nephritis; cerebral infarction right frontal lobe
80278	f	72	1200	05.30	Dec	Epitheliocarcinoma
81032	m	74	1410	20.30	Feb	Cardiac failure; bronchopneumonia
81064	m	83	1280	07.00	Mar	Diverticulitis
82175	m	85	1400	16.45	Jul	Chronic myelocytic leukemia; bronchopneumonia
81100	f	88	1030	00.00	May	Basalioma of ear; amnesic syndrome; cardiac failure
81033	f	90	1110	01.00	Feb	Femur fracture; bronchopneumonia
8538	f	90	1300	09.00	Dec	Non-toxic goitre
83179	f	91	1060	15.30	Jun	Arterial insufficiency; urethral infection

*In nine cases the exact hour of death was not recorded.

which is a major neurosecretory cell group located in the vicinity of the SCN^{22,52,61} was included as a reference nucleus in order to investigate the occurrence of photoperiodic changes in the morphology of other vasopressin-producing cell groups in the hypothalamus. The cytoarchitectonic boundaries of the vasopressin cell populations of both nuclei were delineated by using an antiserum against arginine vasopressin (for details on the immunocytochemistry, see Refs 15,60) (Fig. 1). Only after the measurements had been performed was it established from the records at what moment the subject had died.

Morphometry

Area measurements through the SCN were performed unilaterally by means of a digitizer (Calcomp 2000), using a Zeiss microscope equipped with a 10× (PLAN) objective and 12.5× (PLAN) oculars. In order to describe the shape of the nucleus, the length of the rostrocaudal axis and the surface of the maximum cross-sectional area of the SCN were determined as separate parameters. The length of 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 vasopressin cells were still present. The volume of the vasopressin cell population in the SCN was determined by integrating all sectional areas⁴⁷ that con-

tained immunocytochemically stained cells, taking into account section thickness and the magnification at which the original tracings were made. It means that the SCN, described in the present inquiry, is defined according to immunocytochemical criteria and comprises only a subdivision of the entire nucleus (see Ref. 29). An average of 13 anti-vasopressin stained sections per SCN were measured.

The number of vasopressin neurons in the SCN of each subject was estimated by counting the number of nuclear profiles per unit area in immunocytochemically stained material followed by a discrete "unfolding" procedure⁶⁷ which included a modification proposed by Cruz-Orive¹⁰ and a correction for section thickness. For this purpose, nuclear-profile areas of cells were measured by delineating their outline using the equipment described above with a 40× (PLAN) objective. All labelled cells were counted regardless of the level of immunostaining. The total number of vasopressin neurons in the SCN was estimated by multiplying the numerical cell density with the volume of the vasopressin cell population. The volume and vasopressin cell number of the PVN were determined in the same way at the same side of the brain (for details, see Ref. 23).

Statistics

Subjects were grouped into eight annual periods of equal length (arc length: 45 degrees ~ 1.5 months), based on the time of death, starting at the first of January (Table 2). Differences among groups were statistically evaluated by the

Fig. 1. Photomicrographs of vasopressin-stained frontal sections ($6\ \mu\text{m}$) of the human suprachiasmatic nucleus. A and C show data on a 22-year-old man, who died in October; B and D show data on a 46-year-old woman who died in June. Notice the differences in SCN size and cellular immunoreactivity between these periods of the year. Scale bars = $0.2\ \text{mm}$ (A, B) and $30\ \mu\text{m}$ (C, D).

Table 2. Population characteristics and period of study

Degrees	Time of year Period	No. of subjects	Age (years)	Brain weight (g)	Post mortem delay (h)	Fixation (days)
0-45	1 Jan-15 Feb	8	49.4 ± 8.9	1359 ± 94.6	24.6 ± 10.9	44.4 ± 2.6
46-90	16 Feb-31 Mar	5	51.2 ± 11.3	1326 ± 67.7	22.8 ± 3.0	58.6 ± 31.9
91-135	1 Apr-15 May	2	65.0 ± 23.0	1270 ± 240.0	16.5 ± 5.5	38.5 ± 3.5
136-180	16 May-30 Jun	5	37.6 ± 14.9	1274 ± 85.2	20.8 ± 3.3	52.0 ± 11.3
181-225	1 Jul-15 Aug	6	50.8 ± 8.1	1446 ± 59.5	27.0 ± 6.8	39.5 ± 2.1
226-270	16 Aug-30 Sep	4	55.3 ± 9.2	1337 ± 47.3	15.0 ± 4.2	49.5 ± 2.9
271-315	1 Oct-15 Nov	9	25.1 ± 4.2	1441 ± 77.9	31.2 ± 11.6	34.6 ± 2.3
316-360	16 Nov-31 Dec	9	40.0 ± 9.2	1378 ± 67.2	17.3 ± 2.7	37.0 ± 4.4
Annual mean		48	43.4 ± 3.6	1371 ± 29.1	23.1 ± 3.1	43.0 ± 3.5

Values are given as mean ± S.E.M. Note that none of the parameters measured showed significant differences among the eight periods (P always > 0.2).

Table 3. Seasonal changes in the volume, vasopressin cell number and cell density of the human suprachiasmatic nucleus

Time of year	No. of subjects	Suprachiasmatic nucleus†		
		Volume (mm ³)	Vasopressin cell number (× 10 ³)	Vasopressin cell density (× 10 ³ /mm ³)
1 Jan-15 Feb	8	0.249 ± 0.033	6.68 ± 0.93	27.3 ± 2.3
16 Feb-31 Mar	5	0.301 ± 0.026	7.88 ± 0.92	26.2 ± 2.2
1 Apr-15 May	2	0.217 ± 0.003	6.87 ± 2.73	31.8 ± 13.0
16 May-30 Jun	5	0.140 ± 0.014	3.88 ± 0.43	29.9 ± 5.8
1 Jul-15 Aug	6	0.203 ± 0.028	4.93 ± 1.25	22.9 ± 2.7
16 Aug-30 Sep	4	0.282 ± 0.009	7.57 ± 1.17	26.6 ± 3.4
1 Oct-15 Nov	9	0.351 ± 0.064	10.32 ± 1.66	30.3 ± 3.8
16 Nov-31 Dec	9	0.239 ± 0.044	7.27 ± 1.25	32.3 ± 5.1
Annual mean	48	0.256 ± 0.018	7.17 ± 0.53	28.5 ± 1.5

†Values are given as mean ± S.E.M.

two-tailed Kruskal-Wallis multiple comparisons test.⁹ Since no sexual difference was observed in any of the SCN parameters²³ (also see Refs 22,58), morphometric data were pooled by sex. Throughout this study, values are expressed as means ± S.E.M. A 5% level of significance was used in all statistical tests.

RESULTS

Seasonal variations

A marked seasonal variation was observed in the volume ($P < 0.04$) and the number of vasopressin neurons ($P < 0.05$) of the human SCN (Figs 2,3). The volume of the vasopressin cell population in the SCN was 2.5 times larger in October–November than in May–June ($P < 0.01$) and contained 2.7 times as many vasopressin-immunoreactive neurons ($P < 0.03$) (Table 3).

After the October–November maximum, a reduction in SCN cell number ($P < 0.05$) of about 30% was found in December, after which the number of vasopressin neurons remained relatively stable during the winter period. A further decrease in both vasopressin volume and vasopressin cell number was observed in the period from April through to June. As a result, the seasonal cycle of the human SCN exhibits a non-sinusoidal pattern with a maximum in early autumn, a lower plateau in winter and a deep trough in late spring and early summer. Similar periodic oscillations were found for the total number of cells (including glial cells) in the vasopressin

volume of the human SCN ($P < 0.03$). In contrast, no corresponding annual variations could be detected in vasopressin numerical cell density ($P > 0.9$) or in mean cell–nuclear diameter ($P > 0.7$).

Although the vasopressin volume of the SCN was found to vary significantly over the year, the shape of

Fig. 2. Volume of the vasopressin cell population of the human SCN as a function of time of year. The values represent the mean (± S.E.M.) of data grouped into consecutive 45° intervals (~1.5 months; see Table 2). The seasonal variation in the volume of the SCN is statistically significant (Kruskal-Wallis multiple comparisons test, $P = 0.04$). The vasopressin-containing subdivision of the human SCN is, on average, 2.5 times larger in October–November than in May–June.

Fig. 3. Vasopressin cell number of the human SCN as a function of time of year. The values represent the mean $\pm$ S.E.M. For further details see legend to Fig. 2. The seasonal variation in the cell number of the SCN is statistically significant (Kruskal–Wallis multiple comparisons test, $P = 0.05$). The human SCN contains, on average, 2.7 times as many cells in October–November as in May–June.

the nucleus seems to remain unchanged. However, analysis of the shape of this part of the SCN for males and females separately revealed a significant sexual dimorphism. It appears that the annual cycle in the SCN volume in females is mainly due to changes in the length of the major (rostrocaudal) axis ($P = 0.04$ in females vs $P = 0.15$ in males), whereas measurements in the frontal plane showed that, in males, the largest morphological fluctuations over the year occur in the central part of the nucleus ($P = 0.04$ in males vs $P = 0.28$ in females). In other words, the seasonal variation in the vasopressin volume of the SCN as observed in this study is not due to proportional fluctuations in all parts of the nucleus in the same degree, but affects certain regions of the SCN more than others, depending on the sex of the subject.

It should be noted that the annual variations observed in the human SCN are not the consequence of differences in age, *post mortem* interval or fixation time, since no statistically significant differences were found among the eight annual groups for any of these parameters ($P > 0.2$ at least) (Table 2). Although peptide immunoreactivity may be sensitive to *post-mortem* delays in fixation, neuropeptides are gener-

ally found to be remarkably stable after death.^{48,57} Fliers and co-workers, for example, reported no effect of a *post mortem* interval up to 30 h on vasopressin cell density in the SCN and PVN in humans.^{15,58} A correlation analysis performed in the present study is in agreement with these findings: the number of vasopressin neurons in the SCN was found to be independent of *post mortem* delay ($r = -0.101$, $P > 0.5$) and fixation time ($r = -0.054$, $P > 0.5$). In addition a multi-factor analysis of variance with season, *post mortem* interval and fixation time as independent variables showed that only the time of year has an effect on the number of vasopressin-immunoreactive neurons in the SCN ($F_{7,33} = 29.02$; $P < 0.04$), while both other factors do not have any influence ($P > 0.3$). The PVN, another vasopressin-producing hypothalamic nucleus did not show significant annual variations in the number of vasopressin neurons ($P > 0.2$), although there is a clear tendency in the vasopressin volume of the human PVN to be larger in spring than in other periods of the year (cf. Ref. 24).

Photoperiodic influence

At higher latitudes, as in the Netherlands, where changes in day length are pronounced, light–dark information is the main Zeitgeber for the endogenous annual timing system.¹⁷ To investigate the assumed phase-relationship between the annual variations in the SCN and the dynamic changes in photoperiod, variations in SCN cell number over the year were correlated with the natural photoperiodic conditions at 52° Northern latitude (Fig. 4). The waveform of the SCN cycle shows one peak, with a low amplitude, close to the spring equinox, and a second, more prominent, peak near the vernal equinox, moments of the year when the length of daylight is equal to the length of darkness. Furthermore, the annual minimum was found to coincide with the summer solstice, i.e. the longest photoperiod. These findings indicate that the SCN curve reaches its positive amplitude at the same moment that the photoperiod undergoes its largest rate of change. In other words, large cell numbers in the human SCN are associated with a relatively fast rise and decline in day length.²⁴ It could well be that shortening days in autumn as well as

Table 4. Diurnal changes in the volume, vasopressin cell number and mean cell-nuclear diameter of the human suprachiasmatic nucleus

Time of day (h)	No. of subjects	Volume (mm ³)	Suprachiasmatic nucleus†	
			Vasopressin cell number ($\times 10^3$)	Vasopressin cell–nuclear diameter (μ m)
00.00–06.00	10	0.231 $\pm$ 0.020	6.70 $\pm$ 1.08	6.07 $\pm$ 0.16
06.00–12.00	14	0.299 $\pm$ 0.048	7.68 $\pm$ 1.14	5.89 $\pm$ 0.22
12.00–18.00	7	0.204 $\pm$ 0.031	6.65 $\pm$ 1.19	5.94 $\pm$ 0.34
18.00–24.00	8	0.272 $\pm$ 0.040	7.18 $\pm$ 1.18	5.78 $\pm$ 0.31
Statistics‡		$P > 0.5$	$P > 0.8$	$P > 0.8$
(<i>P</i> -values)				

†Values are given as mean $\pm$ S.E.M.

‡Kruskal–Wallis multiple comparisons test.

Fig. 4. Annual variations in number of vasopressin neurons of the human SCN in relation to the photoperiodic cycle of the temperate zone (52° N). In these double-plotted histograms the SCN data are grouped into 1.5 month periods and expressed as deviations from the annual mean, whereas the photoperiodic deviations from the annual mean are given as a cosine curve. Notice the bimodal waveform of the SCN curve with one maximum close to the spring equinox (March) and the other near the vernal equinox. (September–October).

lengthening days in early spring induce changes in the human SCN. The complexity of the SCN curve with its different amplitudes, however, suggests that the photoperiodic rate is not the only parameter governing this process.

Diurnal variations

The photic influence of the hour of death on the morphology of the hypothalamus was investigated by dividing the 24 h of clock-time into four periods of equal length, starting at 00.00 h. In contrast with the annual cycle of the SCN, no significant diurnal variations in the volume or vasopressin cell number of the human SCN were observed ($P > 0.5$ for both parameters, Table 4). The PVN, like the SCN, did not show diurnal variations, except for the number of vasopressin neurons in the PVN which was found to be about 30% larger in the afternoon (12.00–18.00 h) than in the morning (06.00–12.00 h, $P < 0.04$) or the night (00.00–06.00 h; $P < 0.07$).

Since annual variations in photoperiod may interfere with the diurnal rhythm, and thus, mask possible photic effects of the daily light–dark cycle, a further analysis was performed in which diurnal variations were studied in a stable period (November–February; $315\text{--}45^{\circ}$); i.e. in a period of the year in which photoperiodic changes were found to have only a minor effect on the morphology of the human hypothalamus (Table 3). Also in this period, as in the 12-month period, no significant diurnal variations were observed in the vasopressin cell population in either the SCN ($P > 0.8$) or the PVN ($P > 0.4$). These results, together with the apparent phase relationship between the annual variations in the SCN and the first derivative of the photoperiodic cycle²⁴ indicate that the human vasopressinergic SCN, in contrast with the PVN, appears to be affected by seasonal

variations in photoperiod, and that the daily light–dark cycle as such does not seem to induce comparable morphological changes in the SCN.

DISCUSSION

Human beings are not considered to be particularly photoperiodic, although annual rhythms have been documented in a wide variety of physiological, endocrine and behavioral processes.^{3,45,46} Although social factors may be responsible for a number of these systematic variations over the year, especially in humans, a large body of evidence indicates that the light–dark cycle is the main Zeitgeber for circadian and seasonal rhythms in most species, including man.^{2,3,17} Many of the annual rhythms of human biology are under environmental control, and they may even be driven by an endogenous clock synchronized with the seasons. Several investigations indicate that the SCN and its vasopressin transmitter system are part of the neural substrate that mediates these circannual rhythms (for reviews, see Refs 14,17,33). This hypothesis seems plausible in view of the known involvement of the SCN in the expression of daily, estrous and seasonal cycles.^{32,49,50}

The marked annual variation in the vasopressin cell population of the human SCN, as found in the present study, is in accordance with this view, and with recent findings by Lee and Zucker²⁷ who showed that the SCN is implicated in entrainment and maintenance of normal phase relations of several circannual rhythms in ground squirrels, and that photoperiod appears to act as an effective Zeitgeber for these rhythms. Temperature fluctuations have been implicated in timing annual rhythms in some species.^{39,46} In the present study, however, the variations in the human SCN over the year were found to be independent of the annual temperature cycle ($r = -0.333$; $P > 0.6$). Although temperature cannot be excluded as a potential controlling factor, the present findings suggest that photoperiod is the major Zeitgeber for the observed annual variations in SCN morphology, and that this control is mediated by the retinohypothalamic tract.

The absence of diurnal variations in vasopressin immunoreactivity in the human SCN is in accordance with observations that there are no clear circadian vasopressin patterns measurable in human cerebrospinal fluid, even in cases where normal circadian patterns of rectal temperature and plasma cortisol are present.^{1,55,59} The rather heterogenous character of the subject sample, however, as used in the present study, especially with regard to age, gender, causes of death etc., may have a masking effect on the potential circadian cycle of the vasopressin system in one of the subgroups. Whether this is actually the case remains in need of further investigation. The fact that the morphology of the human SCN shows annual fluctuations, whereas no corresponding diurnal cycle was

found, means that circannual rhythms are probably generated by an oscillator system which differs from the neural circuitry that generates circadian rhythms. A similar uncoupling between seasonal and diurnal rhythms has been reported for some biogenic monoamine levels in the human brain.⁷

The annual variations in vasopressin-immunoreactivity in the human SCN coincide with variations in plasma testosterone level, which is high during late summer and early autumn and low during spring.^{11,41,54} A similar interaction between gonadal hormones and vasopressin immunoreactivity in the brain was reported in various wild living rodents and in laboratory rats.^{6,12,19,20} These observations strongly suggest that, in addition to the sexually dimorphic part of the central vasopressin innervation, the vasopressin system of the SCN is actively involved in the temporal organization of various seasonal functions, including rhythms of reproduction. But not only hormonal levels in humans exhibit annual variations. The amount of sleep per 24-h period also shows a very clear seasonal rhythm.⁶⁸ The least amount of sleep was found in May–June, whereas the largest amount occurred in September–October. It is important to note that these sleep periods correspond with the same periods of the year in which the vasopressin-immunoreactive neurons in the SCN reach their lowest and highest numbers, respectively.

Recent studies have shown that, at least in some mammals, the pineal gland and the suprachiasmatic nucleus are essential for the regulation of photoperiodic responses.^{8,13,16} Although the number of morphometric studies of the pineal gland in mammals in relation to the light–dark cycle and photoperiod is limited (see Refs 30,40) investigations in rodents showed that both the pineal volume and nuclear size of pinealocytes are considerably larger in winter than during the summer period, while the number of “synaptic” ribbons in the pineal, associated with the cyclic biosynthesis of melatonin, have also been found to undergo seasonal variations. Taken together, these results suggest that photic effects on neuropeptidergic activity of the brain are specific to those regions that are directly involved in the transmission of photic information.

CONCLUSIONS

Finally, the more than two-fold difference in the number of vasopressin neurons of the human SCN between late spring and early autumn raises a number of questions about the underlying mechanism. It could be that changes in photoperiod influence the rate of vasopressin synthesis of the SCN and that groups of peptidergic neurons are activated during particular periods of the year, depending on day length, light intensity or other environmental factors. The effects of the pineal hormone melatonin on the metabolic and electrical activity of the mammalian SCN^{8,26} and the distribution of melatonin receptors in the human SCN⁴⁴ suggest that the hormone is involved in temporal organization of this process (for a review, see Ref. 43). Although such a functional interpretation of the annual variations in the SCN sounds plausible, the absence of significant seasonal variations in the numerical density of vasopressin neurons implies that such newly recruited vasopressin cells are not evenly distributed across the entire nucleus. Instead, it might be that the seasonal changes in the SCN are the result of fluctuations in synthetic activity of only those groups of neurons which are located at the periphery of the nucleus. In that case, seasonal changes in the vasopressin expression may indeed have a profound effect on the size and shape of the SCN without affecting the numerical cell density. In other words, the boundaries of the vasopressin cell population in the SCN might be changing because the state, rather than the number, of vasopressin producing neurons may change on a seasonal basis. Further experimental studies should be performed in non-human mammals to examine the exact nature of the seasonally dependent neural plasticity of the biological clock.

Acknowledgements—The authors wish to thank Mr B. Fisser for his technical assistance, Dr P. van der Woude for providing morphometric data on the human PVN, Mr H. Stoffels for drawing the pictures and Ms W. Verweij for her secretarial help. Computer programs for the cell counting procedures were developed by Dr R. W. H. Verwer. Financial support was obtained from Mrs E. J. M. Stevens. Brain material was obtained from the Netherlands Brain Bank, Amsterdam (coordinator Dr R. Ravid).

REFERENCES

1. Amico J. A., Tenicela R. and Robinson A. G. (1985) Neurohypophysial hormones in cerebrospinal fluid of adults: absence of arginine vasotocin and of diurnal rhythm of arginine vasopressin. *J. clin. Endocr. Metab.* **61**, 794–798.
2. Arendt J. and Broadway J. (1987) Light and melatonin as Zeitgebers in man. *Chronobiol. Int.* **4**, 273–282.
3. Aschoff J. (1981) Annual rhythms in man. In *Handbook of Behavioral Neurobiology*, Vol. 4. (ed. Aschoff J.), pp. 475–487. Plenum Press, New York.
4. Beatty W. W. (1979) Gonadal hormones and sex differences in non-reproductive behaviors in rodents: organizational and activation influences. *Horm. Behav.* **12**, 112–163.
5. Binkley S. (1988) *The Pineal, Endocrine and Non-endocrine Function*. Prentice Hall, New York.
6. Buijs R. M., Pévet P., Masson-Pévet M., Pool C. W., De Vries G. J., Canguilhem B. and Vivien-Roels B. (1986) Seasonal variation in vasopressin innervation in the brain of the European hamster (*Cricetus cricetus*). *Brain Res.* **371**, 193–196.
7. Carlsson A., Svennerholm L. and Winblad B. (1980) Seasonal and circadian monoamine variations in human brains examined post mortem. In *Biogenic Amines and Affective Disorders* (eds Svenson T. H. and Carlsson A.) *Psychiat. Scand.* **61**, 75–85.

8. Cassone V. M. (1990) Effects of melatonin on vertebrate circadian systems. *Trends Neurosci.* **13**, 457–464.
9. Conover W. J. (1980) *Practical Nonparametric Statistics*. John Wiley, New York.
10. Cruz-Orive L. M. (1978) Particle shape-size distributions: the general spheroid problem. II Stochastic model and practical guide. *J. Microsc.* **112**, 153–167.
11. Dabbs J. M. (1990) Age and seasonal variation in serum testosterone concentration among men. *Chronobiol. Int.* **7**, 245–249.
12. De Vries G. J., Buijs R. M. and Sluiter A. A. (1984) Gonadal hormones act on the morphology of the vasopressinergic innervation of the adult rat brain. *Brain Res.* **298**, 141–145.
13. Elliott G. A., and Goldman B. D. (1981) Seasonal reproduction: photoperiodism and biological clocks. In *Neuroendocrinology of Reproduction* (ed. Adler N. T.), pp. 377–423. Plenum Press, New York.
14. Follett B. K. and Follett D. E. (1981) *Biological Clocks in Seasonal Reproductive Cycles*. Wright, Bristol.
15. Fliers E., Swaab D. F., Pool C. W. and Verwer R. W. H. (1985) The vasopressin and oxytocin neurons in the human supraoptic and paraventricular nucleus; changes with aging and in senile dementia. *Brain Res.* **342**, 45–53.
16. Goldman B. D. and Darrow J. M. (1983) The pineal gland and mammalian photoperiodism. *Neuroendocrinology* **37**, 386–396.
17. Gwinner E. (1986) *Circannual Rhythms*. Springer, New York.
18. Harrington M. E., Nance D. M. and Rusak B. (1987) Double-labeling of neuropeptide Y-immunoreactive neurons which project from the geniculate to the suprachiasmatic nucleus. *Brain Res.* **410**, 275–282.
19. Hermes M. L. H. J., Buijs R. M., Masson-Pévet M., van der Woude T. P., Pévet P., Brenklé R. and Kirsch R. (1989) Central vasopressin infusion prevents hibernation in the European hamster (*Cricetus cricetus*). *Proc. natn. Acad. Sci. U.S.A.* **86**, 6408–6411.
20. Hermes M. L. H. J., Buijs R. M., Masson-Pévet M. and Pévet P. (1990) Seasonal changes in vasopressin in the brain of the garden dormouse (*Eliomys quercinus* L.). *J. comp. Neurol.* **293**, 340–346.
21. Hoffmann K. (1985) Interaction between photoperiod, pineal and seasonal adaptation in mammals. In *The Pineal Gland. Current State of Pineal Research* (eds Mess B., Ruzsá C., Tima L. and Pévet P.), pp. 221–227. Akadémiai Kiadó, Budapest.
22. Hofman M. A., Fliers E., Goudsmit E. and Swaab D. F. (1988) Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain: sex differences and age-dependent changes. *J. Anat.* **160**, 127–143.
23. Hofman M. A. and Swaab D. F. (1992) Seasonal changes in the suprachiasmatic nucleus of man. *Neurosci. Lett.* **139**, 257–260.
24. Hofman M. A. and Swaab D. F. (1992) The human hypothalamus: comparative morphometry and photoperiodic influences. *Prog. Brain Res.* **93**, 133–149.
25. Kelley D. B. and Pfaff D. W. (1978) Generalizations from comparative studies on neuroanatomical and endocrine mechanisms of sexual behaviour. In *Biological Determinants of Sexual Behavior* (ed. Hutchison J. B.), pp. 225–254. John Wiley, New York.
26. Krause D. N. and Dubocovich M. L. (1990) Regulatory sites in the melatonin system of mammals. *Trends Neurosci.* **13**, 464–470.
27. Lee T. M. and Zucker I. (1991) Suprachiasmatic nucleus and photic entrainment of circannual rhythms in ground squirrels. *J. biol. Rhythms* **6**, 315–330.
28. Lydic R., Schoene W. C., Czeisler C. A. and Moore-Ede M. C. (1980) Suprachiasmatic region of the human hypothalamus: homolog to the primate circadian pacemaker? *Sleep* **2**, 355–361.
29. Mai J. K., Kedziora O., Teckhaus L. and Sofroniew M. V. (1991) Evidence for subdivisions in the human suprachiasmatic nucleus. *J. comp. Neurol.* **305**, 508–525.
30. McNulty J. A., Fox L. M. and Spurrier W. A. (1990) A circannual cycle in pinealocyte synaptic ribbons in the hibernating and seasonal reproductive 13-lined ground squirrel. *Neurosci. Lett.* **119**, 237–240.
31. Meijer J. H. (1990) Physiological basis for photic entrainment. *Eur. J. Morphol.* **28**, 308–316.
32. Meijer J. H. and Rietveld W. J. (1989) Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. *Physiol. Rev.* **69**, 671–707.
33. Mess B. and Ruzsá C. (1986) Relationship between suprachiasmatic nuclei and rhythmic activity of the pineal gland. In *Advances in Pineal Research* (eds Reiter R. J. and Karasek M.), pp. 149–158. John Libbey, London.
34. Mikkelsen J. D. (1990) Projections from the lateral geniculate nucleus to the hypothalamus of the Mongolian gerbil (*Meriones unguiculatus*): an anterograde and retrograde tracing study. *J. comp. Neurol.* **299**, 493–508.
35. Moore R. Y. (1978) The innervation of the mammalian pineal gland. *Prog. reprod. Biol.* **4**, 1–29.
36. Moore R. Y. (1983) Organization and function of a central nervous system circadian oscillator: the suprachiasmatic hypothalamic nucleus. *Fedn Proc.* **42**, 2783–2789.
37. Moore R. Y., Halaris A. E. and Jones B. A. (1978) Serotonin neurons of the midbrain raphe: ascending projections. *J. comp. Neurol.* **180**, 417–438.
38. Moore-Ede M. C., Sulzman F. M. and Fuller C. A. (1982) *The Clocks That Time Us*. Harvard Press, Cambridge, MA.
39. Mrosovsky N. (1986) Thermal effects on the periodicity, phasing, and persistence of circannual cycles. In *Living in the Cold: Physiological and Biochemical Adaptations* (eds Heller H. C., Musacchia X. J. and Wang L. C. H.), pp. 403–410. Elsevier, New York.
40. Peschke E., Schön M., Tertsch S., Peschke D. and Peil J. (1989) Morphometric investigations of the pineal gland after ganglionectomy and thyroectomy under the aspect of circadian and seasonal variations. *J. Hirnforsch.* **30**, 399–407.
41. Reinberg A., Lagoguey M., Cesselin F., Touitou Y., Legrand J.-C. Delassalle A., Antreassian J. and Lagoguey A. (1978) Circadian and circannual rhythms in plasma hormones and other variables of five healthy young human males. *Acta endocr.* **88**, 417–427.
42. Reiter R. J. (1980) The pineal and its hormones in the control of reproduction in mammals. *Endocr. Rev.* **1**, 109–113.
43. Reiter R. J. (1991) Pineal melatonin: cell biology of its synthesis and its physiological interactions. *Endocr. Rev.* **12**, 151–180.
44. Reppert S. M., Weaver D. R., Rivkees S. A. and Stopa E. G. (1988) Putative melatonin receptors in a human biological clock. *Science* **242**, 78–81.
45. Roenneberg T. and Aschoff J. (1990) Annual rhythm of human reproduction. I. Biology, sociology, or both? *J. biol. Rhythms* **5**, 195–216.

46. Roenneberg T. and Aschoff J. (1990) Annual rhythm of human reproduction. II. Environmental correlations. *J. biol. Rhythms* **5**, 217–239.
47. Rosen G. H. and Harry J. D. (1990) Brain volume estimation from serial section measurements: a comparison of methodologies. *J. Neurosci. Meth.* **35**, 115–124.
48. Rossor M., Emson P., Dawbarn D., Dockray G., Mountjoy C. and Roth M. (1986) Postmortem studies of peptides in Alzheimers disease and Huntingtons disease. In *Neuropeptides in Neurologic and Psychiatric Disease* (eds Martin J. B. and Barchas J. D.), pp. 259–277. Raven Press, New York.
49. Rusak B. (1989) The mammalian circadian system: models and physiology. *J. biol. Rhythms* **4**, 121–134.
50. Rusak B. and Zucker I. (1979) Neural regulation of circadian rhythms. *Physiol. Rev.* **59**, 449–526.
51. Sadun A. A., Schaechter J. D. and Smith L. E. H. (1984) A retinohypothalamic pathway in man: light mediation of circadian rhythms. *Brain Res.* **302**, 371–377.
52. Saper C. B. (1990) Hypothalamus. In *The Human Nervous System* (ed. Paxinos G.), pp. 389–413. Academic Press, New York.
53. Shibata S., Liou S. Y., Ueki S. and Oomura Y. (1984) Influence of environmental light-dark cycle and enucleation on activity of suprachiasmatic neurons in slice preparations. *Brain Res.* **302**, 75–81.
54. Smals A. G. H., Kloppenborg P. W. C. and Benraad Th. J. (1976) Circannual cycle in plasma testosterone levels in man. *J. clin. Endocr. Metab.* **42**, 979–982.
55. Sørensen P. S., Hammer M., Gjerris F. and Lundberg J. (1985) 24-h cerebrospinal fluid levels of vasopressin in hydrocephalic patients. *Regul. Pept.* **10**, 115–126.
56. Stopa E. G., King J. C., Lydic R. and Schoene W. C. (1984) Human brain contains vasopressin and vasoactive intestinal polypeptide neuronal subpopulations in the suprachiasmatic region. *Brain Res.* **297**, 159–163.
57. Swaab D. F. (1982) Comments on the validity of immunocytochemical methods. In *Cytochemical Methods in Neuroanatomy* (eds Chan-Palay V. and Palay S. L.), pp. 423–440. Alan R. Liss, New York.
58. Swaab D. F., Fliers E. and Partiman T. S. (1985) The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res.* **342**, 37–44.
59. Swaab D. F., Roozendaal B., Ravid R., Velis D. N., Gooren L. and Williams R. S. (1987) Suprachiasmatic nucleus in aging, Alzheimers disease, transsexuality and Prader-Willi syndrome. *Prog. Brain Res.* **72**, 301–310.
60. Swaab D. F. and Hofman M. A. (1990) An enlarged suprachiasmatic nucleus in homosexual men. *Brain Res.* **537**, 141–148.
61. Swanson L. W. and Sawchenko P. E. (1983) Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *A. Rev. Neurosci.* **6**, 269–324.
62. Turek F. W. (1983) Neurobiology of circadian rhythms in mammals. *BioScience* **33**, 439–444.
63. Turek F. W. (1985) Circadian neural rhythms in mammals. *A. Rev. Physiol.* **47**, 49–64.
64. Turek F. W., Jacobson C. D. and Gorski R. A. (1980) Lesions of the suprachiasmatic nuclei affect photoperiod-induced changes in the sensitivity of the hypothalamic-pituitary axis to testosterone feed-back. *Endocrinology* **107**, 942–946.
65. Turek F. W. and Van Cauter E. (1988) Rhythms in reproduction. In *Physiology of Reproduction* (eds Knobil E. and Neill J. D.), pp. 1789–1830. Raven Press, New York.
66. Underwood H. and Goldman B. D. (1987) Vertebrate circadian and photoperiodic systems: role of the pineal gland and melatonin. *J. biol. Rhythms* **2**, 279–315.
67. Weibel E. R. (1979) *Stereological Methods, Vol. 1, Practical Methods for Biological Morphometry*. London, Academic Press.
68. Wirz-Justice A., Wever R. A. and Aschoff J. (1984) Seasonality in freerunning circadian rhythms in man. *Naturwissenschaften* **71**, 316–319.
69. Zucker I., Boshes M. and Dark J. (1983) Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. *Am. J. Physiol.* **244**, 472–480.

(Accepted 29 October 1992)