Diurnal and Seasonal Rhythms of Neuronal Activity in the Suprachiasmatic Nucleus of Humans

Michel A. Hofman¹ and Dick F. Swaab

Netherlands Institute for Brain Research, Graduate School of Neurosciences, Amsterdam, The Netherlands

Abstract The mammalian suprachiasmatic nucleus (SCN) is considered to be a critical component of a neural system implicated in the temporal organization of a wide variety of biological processes. Since the environmental light–dark cycle is the main zeitgeber for many of these rhythms, photic information may have a synchronizing effect on the endogenous clock in the SCN by inducing periodic changes in the activity of certain groups of neurons. The present study was conducted to investigate whether the daily light–dark cycle as well as seasonal variations in photoperiod would affect the vasopressin cell population of the human SCN. To that end, the brains of 30 young human subjects (ranging in age from 6 to 47 years) were investigated.

We found that the subdivision of the human SCN that contains vasopressin-producing neurons fluctuated significantly over the 24-hr period. The volume of the vasopressin cell population was, on average, 1.4 times as large during the daytime (1000–1800 hr) as during the nighttime (2200–0600 hr), and contained 1.8 times as many vasopressin-immunoreactive neurons. Peak values in both vasopressin volume and vasopressin cell number were observed in the early morning (0600–1000 hr). In general, the SCN contained fewer vasopressin-immunoreactive neurons during the night than during any other period of the natural light–dark cycle.

In addition to the diurnal cycle of the SCN, a marked seasonal rhythm was observed. The volume of the vasopressin cell population was, on average, 2.4 times as large in the autumn as in the summer, and contained 3 times as many vasopressin-immunoreactive neurons. In general, the annual cycle of the human SCN showed a nonsinusoidal pattern with a maximum in early autumn, a lower plateau in winter, and a deep trough in late spring and early summer. In contrast with the periodic fluctuations in the number of vasopressin-immunoreactive neurons in the SCN, no significant diurnal or seasonal variations could be detected in the numerical cell density or cell nuclear diameter of vasopressin neurons.

In conclusion, the findings indicate that the synthesizing activity of the vasopressin neurons of the human SCN exhibits a diurnal as well as a seasonal rhythm, and that the temporal organization of these processes becomes disturbed later in life.

Key words suprachiasmatic nucleus, human hypothalamus, vasopressin neurons, diurnal rhythm, seasonal rhythm, biological clock, photic entrainment

The rotation of the earth results in a periodically changing environment. In such an environment, it would be appropriate for an animal if its internal organization and its behavioral activity could be synchronized with the daily and seasonal fluctuations in illumination, temperature, and humidity, so as to provide an adaptive optimum. The notion that these

1. To whom all correspondence should be addressed, at Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

temporal variations are a significant dimension of biological organization has gained much support over the last two decades (Moore-Ede et al., 1982; Winfree, 1987; Mrosovsky, 1990), and we now have a substantial understanding of the neural mechanisms of a timing system that is responsible for the generation and regulation of circadian rhythms.

The mammalian suprachiasmatic nucleus (SCN), a group of small neurons located in the basal part of the anterior hypothalamus, is generally considered to be the major component of the circadian timing system (for reviews, see Rusak and Zucker, 1979; Moore, 1983, 1991; Turek, 1985; Meijer and Rietveld, 1989). Consistent with the SCN's role in the temporal organization of circadian processes, recent investigations in rodents and nonhuman primates indicate that the SCN is also involved in the seasonal control of reproductive and metabolic phenomena (Follett and Follett, 1981; Zucker et al., 1983, 1991; Turek and Van Cauter, 1988). 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 behavioral processes.

The environmental light-dark cycle is the main zeitgeber in most mammals, including humans (Aschoff, 1981; Gwinner, 1986; Meijer and Rietveld, 1989). Therefore, photic information may have a synchronizing effect on the clock mechanism of the SCN by inducing changes in the functional activity of certain groups of neurons. As in other mammals, the human SCN has distinct populations of peptidergic neurons, which form subdivisions of the nucleus (Mai et al., 1991; Moore, 1992). In recent years, it has become clear that the vasopressin (VP) neurons of the SCN are involved in the transmission of photic information from the outside world to the brain, especially to other hypothalamic areas (Sofroniew and Weindl, 1978; Watts and Swanson, 1987; Watts, 1991; Moore, 1992). Our recent findings seem to support this idea, suggesting that the VP-synthesizing activity of the SCN in human beings fluctuates significantly over the course of the year (Hofman and Swaab, 1992a; Hofman et al., 1993); we found that the volume of the human SCN and the number of VP-containing neurons was about twice as high in the autumn as in the summer. In contrast with the annual cycle, no significant *diurnal* variations in the VP-containing neuron population were observed.

The inability to demonstrate a circadian pattern in the number of VP-immunoreactive neurons in the SCN may be attributable to a lack of synchrony in circadian rhythmicity of the VP-synthesizing activity in elderly subjects. In fact, it is widely believed that the circadian timing system is progressively disturbed in senescence, both in humans and in other mammals, as demonstrated by a reduced amplitude and period length of circadian rhythms and by an increased tendency toward internal desynchronization (for reviews, see Miles and Dement, 1980; Brock, 1985; Van Gool and Mirmiran, 1986; Richardson, 1990). In humans, age-related changes have been described for hormonal rhythms, body core temperature, and the sleep-wake cycle, as well as for several other behavioral cycles (see, e.g., Mirmiran et al., 1989; Cornélissen et al., 1992; Touitou and Haus, 1992). In the present study, therefore, we investigated the diurnal and seasonal rhythmicity of the VP neuron population of the human SCN in young subjects.

METHODS

SUBJECTS

Brains of 30 young human subjects (18 males and 12 females), ranging in age from 6 to 47 years (mean $\pm$ SEM = 27.3 $\pm$ 2.2 years), were obtained at autopsy (Table 1). These

TABLE 1. Brain Material

Patient	Sex	Age	Hour of death ^a	Month of death	Postmortem delay (hr)	Fixation time (days)	SCN		Clinical diagnosis
							VP volume (mm ³)	VP cell number ($\times 10^5$)	
30587	M	6	0800	Oct	4	41	0.490	11.31	Intestinal necrosis and peritonitis
87378	F	7	0715	Nov	17	33	0.734	18.27	Astrocytoma; brain edema
86870	F	9	—	May	24	91	0.153	3.84	Cytotoxic brain edema
81306	F	10	0900	Dec	16	32	0.525	11.90	Cardiac failure; bronchopneumonia; sepsis
87191	F	13	0400	Jun	24	48	0.186	2.51	Histiocytic lymphoma; hemorrhagic diathesis
87444	M	14	—	Dec	24	27	0.286	7.08	Congenital myopathia; cardiac failure
80280	F	15	1300	Dec	20	29	0.309	4.73	Cerebellar hematoma; lung edema
81043	M	16	1800	Feb	17	49	0.428	10.09	Multiple traumata; cerebral contusion
81241	M	19	0030	Oct	9	34	0.231	8.41	Retropitoneal chondrosarcoma; septic shock
83306	M	22	1815	Oct	15	35	0.380	12.93	Lymphatic leukemia, cerebral edema
87110	M	23	1100	Mar	13	11	0.379	9.92	Encephalitis in brainstem
87626	M	27	2300	Feb	40	28	0.129	3.45	Coma; cerebral edema
82181	M	27	—	Jul	24	40	0.196	3.97	Drug addiction; sepsis (<i>S. aureus</i>)
81058	M	28	1000	Mar	23	32	0.303	5.45	Medial cerebral artery aneurysm; lung emboli
86414	M	28	—	Nov	24	46	0.233	9.89	Guillain-Barré syndrome; bronchopneumonia
85124	F	29	2045	May	24	60	0.107	5.16	Alcoholic cirrhosis; hemorrhagic ascites
84186	M	29	2130	Aug	13	41	0.279	5.24	Congenital heart disease; cardiac failure
81255	F	30	1145	Nov	24	39	0.159	4.44	Intramural dissecting haematoma of coronary artery
81251	M	31	2015	Nov	29	30	0.339	9.25	Multiple traumata; small subarachnoid hemorrhage
86354	F	33	—	Oct	24	20	0.229	12.70	Adenocarcinoma; multiple metastases
80271	F	35	0500	Dec	8	36	0.206	4.40	Acute lymphoblastic leukemia
84018	F	36	2100	Jan	96	51	0.271	4.01	Multiple fractures; rupture of thoracic aorta
84248	M	37	0130	Oct	39	35	0.133	1.87	Bronchopneumonia
87260	M	37	—	Jul	48	46	0.169	2.95	Alcohol intoxication, combined with benzodiazepines
81012	F	38	1215	Jan	3	47	0.229	8.33	Cervix carcinoma
87345	M	41	—	Oct	120	44	0.464	13.73	Cerebral contusion; lung emboli
81093	M	42	1115	Apr	22	42	0.214	9.60	Metastatic bronchogenic carcinoma; pneumothorax
81267	M	43	1230	Nov	23	53	0.195	10.75	Non-Hodgkin lymphoma; sepsis
83173	F	46	0615	Jun	11	33	0.117	4.23	Adrenal carcinoma; postoperative hemorrhage
87271	M	47	—	Jul	24	39	0.138	2.24	Amyotrophic lateral sclerosis

^a In eight cases, the exact hour of death was not recorded.

brains, which were from individuals who had no known neurological or psychiatric disorders, were collected and neuropathologically examined in either the Free University Hospital (Dr. W. Kamphorst) or the Academic Medical Center (Dr. D. Troost), both in Amsterdam, The Netherlands (latitude 52°N), during the period 1981–1987. The present set of data is a subset of a larger cohort, which included both young and elderly subjects and which has been used in previous studies on seasonal variations in the human hypothalamus (see Hofman et al., 1993). The times of death of the subjects were uniformly distributed over the 24-hr period as well as over the year (Rayleigh test; $p > 0.2$ for both periods). Only those subjects whose actual death times were known were included in the diurnal analysis. In eight cases, the exact hour of death was not recorded.

HISTOLOGY

Brains were removed from their skulls within 48 hr postmortem, weighed, and immediately placed into 4% formaldehyde, in which they were stored at room temperature for about 1 month prior to histological preparation (Table 1). Within this period, the brain, after a considerable initial swelling, returns approximately to its fresh volume (Bauchot, 1967). The hypothalamic area containing the SCN was dissected from each brain by frontal sections rostrally of the optic chiasm and caudally of the corpora mammillaria, and was subsequently dehydrated in graded ethanol and embedded in paraffin. It should be noted that although embedding in paraffin provokes a correlated shrinkage of the tissue, histological artifacts did not in any way affect the general outcome of the present study. The reason for this is that not only the volume of the VP cell population in the SCN was measured, but also its VP cell number, which is independent of the preparation method or staining procedure. Serial 6- μ m frontal sections were cut on a microtome, mounted on slides coated with chrome and aluminum sulfate, deparaffinized, hydrated, and brought to phosphate-buffered saline. For general orientation purposes, sections were stained with thionin. The cytoarchitectonic boundaries of the VP-producing cell population of the SCN were delineated by using an antibody against arginine VP as marker, and every 25th section was stained (for details on the immunocytochemistry, see Swaab and Hofman, 1990). Only after the measurements had been performed was it established from the records at what moment each subject had died.

MORPHOMETRY

Cross-sectional area measurements through the SCN were performed unilaterally, mainly on the right-hand side, by means of a Calcomp 2000 digitizer connected to an HP 9000/835 computer, using a Zeiss microscope equipped with a $\times 10$ (PLAN) objective and $\times 12.5$ (PLAN) oculars. 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 VP cells were still present. Scattered parvocellular VP-immunoreactive neurons that were sometimes found at considerable distances from the cell-dense part of the SCN were not included in the measurements. The volume of the VP cell population in the SCN was determined by integrating all sectional areas that contained immunocytochemically stained cells, taking into account section thickness and the magnification at which the original tracings were made. This meant that the SCN, as described in the present inquiry, was defined according to immunocytochemical criteria and comprised only a subdivision of the entire nucleus (see Mai et al., 1991). We measured an average of 13 anti-VP-stained sections per SCN.

The number of VP 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 deconvolution procedure (Weibel, 1979), which included a modification proposed by Cruz-Orive (1978) 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 $\times 40$ (PLAN) objective. All labeled cells were counted, regardless of the level of immunostaining. The total number of VP neurons in the SCN was estimated by multiplying the numerical cell density by the volume of the VP cell population.

DATA ANALYSIS

Subjects were grouped into four diurnal periods and four annual periods, based on the times of their deaths (see Tables 2 and 3, below). Since it was difficult to subdivide the daily light–dark cycle into a “dark” and a “light” period, especially for hospital patients who were living under seminatural lighting conditions, we decided to divide the 24-hr day into four periods, taking into consideration the hospitals’ time schedule as well as the annual variations in photoperiod. Therefore, two transitional periods (“dawn” and “dusk”), whose “light” versus “dark” status was variable, were dealt with separately. At 52°N, photoperiods range from 7 hr and 45 min at the time of the winter solstice, to 16 hr and 44 min at the time of the summer solstice. It should be noted that our seasonal subdivision was based on a “photoperiodic” division of the year, which means that the seasons referred to here do not correspond with the conventional “meteorological” division.

Differences among groups were evaluated statistically by the Kruskal–Wallis multiple-comparisons test. To determine the effects of both clock time and season on the SCN morphology, data were analyzed using two-factor analysis of variance (ANOVA). Since a two-factor data array with unequal subclass sizes and missing values can give rise to special analytical problems, the nonparametric Durbin test for incomplete block designs was performed as well (Conover, 1980; see also Kleinbaum et al., 1988). Because no sexual difference was observed in any of the SCN VP cell parameters (Hofman and Swaab, 1992a; see also Swaab et al., 1985; Hofman et al., 1988), morphometric data were pooled by sex. The diurnal and annual patterns in SCN VP cell number were examined by applying a scatterplot smoothing procedure with an equally weighted moving average ($\delta h = 45$ degrees; $0.45 < f < 0.6$) (cf. Cleveland, 1985). A smoothing procedure was applied to reduce the effect of random error and to enhance the nonlinear, periodic pattern of the synthesizing activity of the VP neurons. Throughout this paper, values are expressed as mean $\pm$ standard error of the mean (*SEM*). A significance level of 5% was used in all statistical tests.

RESULTS

DIURNAL VARIATIONS

The subdivision of the human SCN containing VP-producing neurons showed a remarkable diurnal variation (Table 2). The volume of the VP cell population was, on average, 1.4 times as large during the daytime as during the nighttime ($0.255 \pm 0.029 \text{ mm}^3$ and $0.177 \pm 0.020 \text{ mm}^3$, respectively), and contained 1.8 times as many VP-immunoreactive neurons

TABLE 2. Diurnal Variations in the VP Cell Population of the SCN in Young Subjects (<50 Years of Age)

Time of day	Period (hr)	No. of subjects	SCN VP cell population variables ^a		
			Volume (mm ³)	Cell number ($\times 10^3$)	Cell nuclear diameter (μ m)
Dawn	0600–1000	4	0.467 $\pm$ 0.128	11.43 $\pm$ 2.87	5.97 $\pm$ 0.70
Daytime	1000–1800	7	0.255 $\pm$ 0.029	7.60 $\pm$ 1.01	5.76 $\pm$ 0.28
Dusk	1800–2200	6	0.301 $\pm$ 0.046	7.78 $\pm$ 1.43	5.74 $\pm$ 0.31
Nighttime	2200–0600	5	0.177 $\pm$ 0.020	4.13 $\pm$ 1.15	5.96 $\pm$ 0.33
Statistics ^b			$p = 0.129$	$p = 0.053$	$p = 0.928$

^a Values are represented as means $\pm$ SEM.^b Kruskal–Wallis multiple-comparisons test.

(7.60 $\pm$ 1.01 $\times 10^3$ and 4.13 $\pm$ 1.15 $\times 10^3$, respectively) (Tables 2 and 4). In general, the human SCN contained fewer VP-producing neurons during the night (2200–0600 hr) than during any other period of the natural light–dark cycle ($p \leq 0.05$). Peak values in both VP volume and VP cell number were observed in the early morning (0600–1000 hr)—that is, during the transition from dark to light (Fig. 1 and Table 2). In contrast, no corresponding diurnal variations could be detected in VP numerical cell density ($p > 0.6$) or in mean cell nuclear diameter ($p > 0.9$). The VP cell population of the paraventricular nucleus of the hypothalamus, another VP-producing cell group located in the vicinity of the SCN, did not show any significant diurnal variations.

Since seasonal variations in the VP neuronal population of the SCN may interfere with the diurnal rhythm (see next section), a further analysis was performed in which diurnal variations were studied by excluding the low summer values from the data set. As a result, the within-group variance diminished and a more prominent diurnal rhythm appeared in both the VP volume of the SCN ($p = 0.006$) and the number of VP neurons ($p = 0.025$), with the values in the light period (0600–2200 hr) being, on average, about twice as high as those in the dark period (2200–0600 hr).

TABLE 3. Seasonal Variations in the VP Cell Population of the Human SCN in Young Subjects (<50 Years of Age)

Time of year	Period (degrees)	No. of subjects	SCN VP cell population variables ^a		
			Volume (mm ³)	Cell number ($\times 10^3$)	Cell nuclear diameter (μ m)
Spring	36°–125° midpoint: Mar 23	5	0.291 $\pm$ 0.054	7.70 $\pm$ 1.37	5.57 $\pm$ 0.35
Summer	126°–215° midpoint: Jun 22	7	0.152 $\pm$ 0.013	3.56 $\pm$ 0.39	5.51 $\pm$ 0.31
Autumn	216°–305° midpoint: Sep 21	8	0.368 $\pm$ 0.068	10.56 $\pm$ 1.83	5.52 $\pm$ 0.28
Winter	306°–35° midpoint: Dec 21	10	0.275 $\pm$ 0.033	7.48 $\pm$ 0.93	5.92 $\pm$ 0.31
Statistics ^b			$p = 0.010$	$p = 0.009$	$p = 0.747$

^a Values are represented as means $\pm$ SEM.^b Kruskal–Wallis multiple-comparisons test.

TABLE 4. Effect of Light Intensity (Day versus Night) and Photoperiod (Summer versus Winter) on the VP Cell Population of the Human SCN in Young Subjects (< 50 Years of Age)

SCN VP cell population variable	Daytime/nighttime ^a	Significance ^b	Winter/summer ^a	Significance ^b
Volume	1.44	$p = 0.088$	1.81	$p = 0.003$
Cell number	1.84	$p = 0.028$	2.10	$p = 0.003$
Numerical cell density	1.43	$p = 0.223$	1.14	$p = 0.435$
Cell nuclear diameter	0.97	$p = 0.465$	1.07	$p = 0.435$

^a Values are given as ratios of means. Daytime, 1000–1800 hr; nighttime, 2200–0600 hr; winter, 306°–35°; summer, 126°–215°; see also Tables 2 and 3.

^b Wilcoxon/Mann–Whitney U test.

SEASONAL VARIATIONS

A marked seasonal cycle was observed in the VP volume of the SCN ($p = 0.010$) and in the number of VP neurons ($p = 0.009$). The volume of the VP cell population was, on average, 2.4 times as large in the autumn as in the summer ($0.368 \pm 0.068 \text{ mm}^3$ and $0.152 \pm 0.013 \text{ mm}^3$, respectively), and contained three times as many VP-immunoreactive neurons ($10.56 \pm 1.83 \times 10^3$ and $3.56 \pm 0.39 \times 10^3$, respectively) (Table 3). In general, the human SCN was smaller and contained fewer VP-producing neurons in the summer than in any other period of the year (Fig. 2). Although peak values were found in the autumn, the number of VP neurons in the SCN of patients who had died in the winter was still more than twice as high as that in the SCN of patients who had died in the summer. In contrast, no such seasonal variations could be detected in VP numerical cell density ($p > 0.7$) or in mean cell nuclear diameter ($p > 0.7$; see Table 3).

In previous studies (Hofman and Swaab, 1992a; Hofman et al., 1993), in which no distinction was made between young and elderly subjects, we found a similar though less prominent seasonal pattern in the cellular morphology of the SCN. It seems as if the amplitude and/or phase of the annual cycle of the VP-producing system of the human SCN changes

FIGURE 1. Diurnal rhythm in the number of VP-containing neurons in the human SCN in young subjects (<50 years of age). The black bars indicate the night period (2200–0600 hr). The general trend in the data was enhanced by using a smoothed, double-plotted curve (see “Data Analysis”). The line represents mean values; the shaded area around the line represents SEMs.

FIGURE 2. Annual rhythm in the number of VP-containing neurons in the human SCN in young subjects (<50 years of age). The general trend in the data was enhanced by using a smoothed, double-plotted curve. For further details, see legend to Figure 1.

in later life. This means that incorporation of data from aged subjects into the earlier data set may have had a masking effect on cyclic phenomena and reduced the possibility of detecting periodic variations in SCN neuronal activity.

The number of VP-containing neurons in the paraventricular nucleus did not show any significant changes over the year ($p > 0.9$). Yet there is a clear tendency in the VP volume of the human paraventricular nucleus to be larger in spring and autumn than in summer and winter (see Hofman et al., 1993), which is attributable mainly to seasonal variations in neuronal density. In spring and autumn, the VP neurons of this nucleus appear to be less densely packed than in the other seasons.

To test the hypothesis that both clock time and season would have an effect on the number of VP-immunoreactive neurons, a two-factor ANOVA was performed. The analysis showed that both factors had a significant influence on the VP cell population of the human SCN, and that there was no interaction effect, $F(6, 9) = 0.36$, $p > 0.8$. Similar results were obtained using the nonparametric Durbin test for incomplete block design (see "Data Analysis"). In other words, the effect of clock time on the peptidergic activity of the VP neurons was independent of the seasonal effect.

DISCUSSION

The mammalian SCN is considered to be the major component of the biological clock, involved in the temporal organization of a wide variety of physiological and behavioral circadian rhythms. Consistent with its role in the circadian organization of mammals, recent investigations suggest that measurable biological activities within the SCN, such as glucose utilization, neuronal electrical activity and protein synthesis, exhibit circadian rhythms (for reviews, see Gillette, 1991; Newman, 1991; Schwartz, 1991). Tominaga et al. (1992), for example, demonstrated that under normal light-dark conditions, VP levels in the rat SCN showed circadian rhythmicity, with a peak at the early light phase and a broad trough during the dark phase (see also Noto et al., 1983; Södersten et al., 1985; Yamase et al., 1991).

This circadian pattern in the VP content was maintained even in constant darkness and is similar to that of somatostatin, another peptide in the dorsomedial SCN. In contrast, two other neuropeptides in the ventrolateral SCN, vasoactive intestinal polypeptide and gastrin-releasing peptide, did not show endogenous circadian variations in constant darkness (see Shinohara et al., 1993).

The present findings are in accordance with these experimental studies and indicate that the number of VP-immunoreactive neurons in the human SCN also exhibits diurnal fluctuations. A peak in the number of VP-producing neurons was observed in the early morning, the same period in which the release of VP in rat SCN slices reaches its highest level (Earnest and Sladek, 1986; Gillette and Reppert, 1987). It is also interesting to note that the only identified gene product that is regulated in a circadian fashion within the SCN—the messenger RNA for VP—has been reported to be higher during the day than during the night (Uhl and Reppert, 1986; Majzoub et al., 1991).

Experimental studies carried out in laboratory animals have also indicated that the circadian rhythmicity is disrupted during senescence at various levels of biological organization (for reviews, see Van Gool and Mirmiran, 1986; Richardson, 1990). In analogy with these data, many human circadian rhythms have been reported to be decreased or lost with normal aging (Miles and Dement, 1980; Mirmiran et al., 1989; Touitou and Haus, 1992); this is believed to be the result of age-related changes in the functional activity and cytoarchitectonic organization of the biological clock (Swaab et al., 1988, 1993; Mirmiran et al., 1989; Hofman and Swaab, 1993). The occurrence of degenerative changes in the circadian timing system during aging may be the reason why in our earlier studies, in which no distinction was made between young and old subjects (Hofman and Swaab, 1992a; Hofman et al., 1993), no significant diurnal variations in the VP system of the SCN could be detected. The present findings, however, clearly demonstrate that there is a diurnal rhythm in the VP cell population of the human SCN, at least in nonelderly individuals. Because the number of VP-immunoreactive neurons probably reflects the peptidergic activity state of the neurons, these results suggest that the synthesis of VP in the human SCN exhibits a circadian rhythmicity that is entrained to the environmental light–dark cycle.

Since many VP-containing neurons of the SCN project to areas outside the SCN, mainly toward the paraventricular region of the hypothalamus (Swanson and Cowan, 1975; Berk and Finkelstein, 1981; Watts and Swanson, 1987), we also looked for diurnal and seasonal changes in the VP-immunoreactive neuron population of the paraventricular nucleus. Contrary to what was expected, the VP system of the paraventricular nucleus did not show any periodical fluctuations in neuronal activity, despite the fact that this region is a major integrative center concerned with neuroendocrine, autonomic, and behavioral circadian processes (see Swanson, 1987; Cunningham and Sawchenko, 1991). Although we failed to detect a diurnal rhythm in the paraventricular nucleus, the possibility remains that not all VP neurons, but only a specific subdivision of the VP-producing neurons, exhibits a circadian pattern. In particular, the parvocellular regions of the paraventricular nucleus in some rodent species appear to receive an input from the SCN and peri-SCN region by which photic information is relayed to the pineal gland (Reiter, 1980; Swanson and Kuypers, 1980; Moore, 1983; Pickard and Turek, 1985; Rusak, 1989; Reuss et al., 1990; Illnerová, 1991; Watts, 1991). Therefore, it would be interesting to investigate whether the VP synthesis of this particular cell group in the human paraventric-

ular nucleus exhibits a diurnal rhythm. In rodents, however, these neuronal populations form discrete subnuclei; by contrast, the subdivisions of the paraventricular nucleus in the human brain are not clear-cut (Gai et al., 1990; Saper, 1990). This makes it difficult to discriminate the magnocellular VP-ergic neurons, the efferents of which terminate in the pituitary gland, from the parvocellular neurons that are thought to be an important component of the SCN–pineal complex. Other factors—such as the high stainability of VP neurons in the paraventricular nucleus, which hinders immunocytochemically stained cells from falling below detection level—may also explain why we were unable to find a neuronal rhythmicity in this hypothalamic cell group.

Although human beings are not considered to be particularly photoperiodic, many of the annual rhythms of human biology are under environmental control, and seem to be driven by an endogenous clock synchronized with the seasons. In recent years, it has become clear that in mammals the pineal gland and the SCN are essential for the regulation of these annual rhythms (for reviews, see Follett and Follett, 1981; Mess and Rzss, 1986; Zucker et al., 1991). The marked seasonal variation in the VP cell population of the human SCN, as found in the present study, is in line with this view; it also agrees with recent findings by Lee and Zucker (1991), 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.

In analogy, we recently found a phase relationship between the annual variations in the human SCN and the natural photoperiodic cycle (Hofman and Swaab, 1992b; Hofman et al., 1993). The findings suggest that shortening days in autumn, as well as lengthening days in early spring, induce changes in the VP synthesis of the SCN. This does not necessarily imply that the SCN is the “master” clock for the generation and expression of circannual rhythms, as it probably is for the circadian timing system (Meijer and Rietveld, 1989; Moore, 1992). It may well be that circannual rhythms are generated by an oscillator system differing from the neural circuitry that generates circadian rhythms. It has been shown, for example, that in ground squirrels some brain lesions that eliminated circadian rhythm expression spared circannual rhythms, whereas other neural insults disrupted circannual but not circadian cycles (Zucker et al., 1983; Dark et al., 1985; see also Gwinner, 1981; Zucker, 1988). These results suggest that the generation and expression of circannual rhythms are not dependent on the integrity of the circadian system. The complexity of the seasonal variations in the SCN also suggest that, in addition to the environmental lighting conditions, other, nonphotic signals are involved in generating seasonal cycles. There is now substantial evidence that the SCN and pineal gland are involved in a neuroendocrine feedback loop (see, e.g., Reiter, 1980; Goldman and Darrow, 1983; Mess and Rzss, 1986; Pvet, 1987; Underwood and Goldman, 1987; Cassone, 1990). Gonadal hormones probably also mediate the period, phase, and coherence of activity rhythms that occur in association with natural reproductive cycles, including estrous cycles and photoperiodic responses (Morin and Cummings, 1981; Gwinner, 1986; Turek and Van Cauter, 1988). Thus, a neuroendocrine substrate underlying circannual interactions is a distinct possibility. Further experimental studies should be performed in nonhuman mammals to examine the exact nature and location of the circannual clock and the means by which it is synchronized to the annual geophysical cycle.

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