Journal of Neuropathology and Experimental Neurology

Vol. 58, No. 1
January, 1999
pp. 29–39

Pathologic Evaluation of the Human Suprachiasmatic Nucleus in Severe Dementia

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Abstract. Sleep disruption and other circadian rhythm disturbances are frequently seen in dementia patients. In this study, we examined the suprachiasmatic nucleus (SCN), the putative site of the hypothalamic circadian pacemaker, to determine the nature and degree of pathologic changes caused by *severe* dementia. Neuropathologic examination indicated that among 30 patients with a clinical history of severe dementia, 22 had Braak and Braak stage V–VI Alzheimer disease, 3 had combined Alzheimer and Parkinson disease, 3 had Pick disease and 2 had severe hippocampal sclerosis. Comparisons were made with a control group composed of 13 age-matched patients with no clinical or pathological evidence of dementia or other CNS disorders. To determine the pathologic involvement within the SCN, human hypothalami were stained with: Nissl, Bielchowsky silver, thioflavin S and specific antibodies directed against vasopressin (VP), neurotensin (NT), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), beta-amyloid (B/A4) and glial fibrillary acidic protein (GFAP). Pathologic damage was primarily limited to neuronal loss and neurofibrillary tangle formation. Only rare diffuse plaques were noted. The pathologic changes within the SCN were less severe than in the other brain regions. Morphometric analysis was accomplished using a stereological approach to sample the average total number of positively stained neurons and astrocytes in 10 different 0.1mm^2 microscopic fields in the dorsal subdivision of the SCN. Patients with Alzheimer disease exhibited a significant decrease in vasopressin (9.75 vs 16.7, $p < 0.001$) and neurotensin (6.82 vs 9.63, $p < 0.002$) neurons, as well as a corresponding increase in the GFAP-stained astrocyte/Nissl-stained neuron ratio (0.54 vs 0.10, $p < 0.009$). These studies provide evidence that both vasopressin and neurotensin neurons are lost in Alzheimer disease, and that the astrocyte/neuron ratio is a reliable indicator of disease-related pathology within the SCN. Taken collectively, our data support the hypothesis that damage to the SCN may be an underlying anatomical substrate for the clinically observed changes in circadian rhythmicity that have been observed in Alzheimer patients.

Key Words: Alzheimer disease; Circadian rhythms; Human brain; Hypothalamus; Neuropeptides; Suprachiasmatic nucleus.

INTRODUCTION

Alzheimer Disease (AD) is commonly associated with changes in normal patterns of behavior in addition to decline in cognitive function. Hyperactivity and disorders of the sleep-wake cycle occur in many AD patients. Some exhibit severe restlessness characterized by nearly constant walking and an inability to remain sitting if unrestrained (1). Other AD patients develop "sundowning," a syndrome occurring in the late afternoon or early evening that is clinically indistinguishable in its cognitive and behavioral manifestations from delirium (2). Fluctuations in the level of cognitive functioning may occur during the day or from one day to the next in AD patients in the absence of discernible cause (1).

Changes in the circadian rhythm of core-body temperature have been found with normal aging, including reduced rhythm amplitude, a phase advance in the timing of the nocturnal temperature minimum, and a shortened intrinsic period (3–6). The handful of published studies of the temperature cycle in AD patients are not consistent with regard to amplitude and phase, but suggest that the normal temperature rhythm may be influenced by the disease (7–11).

An extensive body of experimental evidence indicates that the suprachiasmatic nucleus (SCN) in the anterior hypothalamus is the site of the principal circadian oscillator in mammals (12, 13). Clinical observations in humans also support the hypothalamic regulation of circadian functions. Von Economo noted that the lesions of the anterior hypothalamus seen in encephalitis lethargica were associated with disruption of the circadian sleep cycle (14), and patients with hypothalamic tumors have been reported to have body temperature and sleep-wake disturbances (15, 16).

Studies of the SCN in humans, however, have been relatively few in number, and some early investigators even questioned the existence of the SCN in human hypothalamus (17, 18). Most recent human studies support the presence of a suprachiasmatic nucleus that is structurally organized in a manner similar to other mammals (19–25). Some structural differences between the human SCN and the SCN of other species have been reported, including a large neurotensin-containing neuronal subpopulation and neuropeptide Y—containing neuronal cell bodies (22, 23, 26). Like the SCN in other mammals, the

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Supported by AG09301, AG10682, and Harvard Brain Tissue Resource Center MH/NS 31862.

human SCN has been shown to have specific receptors for melatonin, supporting the existence of a pineal feedback loop (27). A human retinohypothalamic tract (RHT) has also been identified, further suggesting that human circadian organization resembles other mammals (28–30).

Despite general agreement that an SCN is present in human hypothalamus, descriptions of the boundaries of the SCN have varied depending on the staining technique(s) employed. For example, the boundaries of the SCN observed with classical Nissl staining differ from those defined by immunohistochemical staining of the 3 predominant cell types, the vasopressin (VP), vasoactive intestinal peptide (VIP), and neurotensin (NT) containing neuronal subpopulations (20, 22). Delineation of the boundaries of the SCN in humans may also be complicated by other factors such as variation in the cell density of the human pre-optic region and inter-patient variation in brain size and shape. Morphometric studies of the SCN based on the distribution of the VP-containing neuronal sub-population have indicated that the size and shape of the nucleus may vary with gender, aging, sexual preference, season, time of day, and various disease states (21, 31–38). Changes in the size and shape of the VIP-containing neuronal subpopulation appear to differ from those observed for the VP-containing neurons (39–41).

In the present study, we focus on defining the specific neuropathologic changes within the SCN that result from severe dementia. In order to improve our ability to detect subtle pathologic alterations, we have purposely limited our study to patients with advanced disease. Finally, a novel stereological approach aimed at sampling the pathologic alterations occurring within the dorsal subdivision of the SCN has been devised.

MATERIALS AND METHODS

Subjects

All dementia brains (n = 30) were obtained at autopsy from male inpatients on the Dementia Study Unit at the Bedford VA Hospital (mean postmortem interval = 15 hours (h); mean age ± standard deviation = 72 ± 8). These patients all met NINCDS-ADRDA criteria for probable AD (42) and had severe cognitive impairment that prevented them from being tested with any formal scale of cognitive functioning. They were unable to communicate verbally, to assist with activities of daily living, or to ambulate. They remained in bed, with all of their needs regularly attended to by nursing staff. All dementia patients expired as a result of complications related to their chronic disease (pneumonia, aspiration, cachexia, etc.). The mean duration of dementia was approximately 10 years.

Control brains (n = 13) were obtained from aged male hospital patients with no prior history of dementia or affective illness and no pathologic evidence of AD or other degenerative brain diseases (mean postmortem interval = 12 h; mean age ± standard deviation = 63 ± 12). The causes of death included: myocardial infarction (4), congestive heart failure (3), colon cancer (1), lung cancer (2), renal failure (1), pneumonia (1), and adult respiratory distress syndrome (1).

Neuropathologic Examination

All brains were subjected to a standardized neuropathologic examination. Brains were fixed for at least 4 weeks in 10% neutral buffered formalin to standardize shrinkage during fixation. Following gross examination and coronal sectioning, the hypothalamus was dissected from the specimen for special studies of the suprachiasmatic nucleus. Fourteen brain areas were routinely sampled in our protocol. These representative areas were carefully selected on the basis of their suitability for diagnosing AD, as well as the other currently classified neurodegenerative diseases. The diagnosis of AD was made in accordance with widely accepted NIA criteria (43) as well as the Braak and Braak neuropathological staging of Alzheimer-related change (44). The diagnosis of Pick disease was based on the presence of severe frontal and temporal lobe atrophy with neuronal loss, gliosis, and Pick bodies within the hippocampus and neocortex. The diagnosis of Parkinson disease required evidence of gliosis, pigment incontinence, and multiple Lewy bodies in the substantia nigra and locus coeruleus. Hippocampal sclerosis was diagnosed in patients who demonstrated extensive neuronal loss and gliosis within the hippocampus, but had no evidence of an underlying degenerative disease. Both of these patients also had evidence of arteriolar sclerosis.

SCN Studies

The pathologic effects of Alzheimer disease on the entire human SCN were examined in AD (n = 3) and control (n = 3) hypothalami. For these studies, hypothalami were immediately immersed in 4% paraformaldehyde at the time of autopsy and subsequently cryoprotected in 0.1 M sodium phosphate buffer containing 30% sucrose. Serial cryostat sections through the anterior hypothalamus were made at 40 microns and alternately stained with Nissl, thioflavin S, Bielschowsky, and antibodies directed against B/A4, VP, NT, NPY, and VIP.

For quantitative morphometric analyses, which focused specifically on the dorsal subdivision of the human SCN, routinely processed formalin fixed paraffin embedded hypothalami from AD (n = 27) and control (n = 10) patients were serially sectioned at 8 microns in the coronal plane. Previous studies have indicated that the dorsal subdivision has the greatest overall diameter and cell density, and is easily recognized between the lateral border of the supraoptic recess of the third ventricle and the supraoptic nucleus (20, 22–23). This dorsal subdivision is predominantly composed of VP and NT neurons. Unlike other SCN peptides (e.g. VIP), which require specialized fixation for optimal detection, the vasopressin and neurotensin neurons are easily identified by immunohistochemistry performed on routinely processed formalin-fixed, paraffin-embedded brain tissue.

The stereological methods utilized in our studies were designed in accordance with the principles described by Weibel for random tissue sampling of a homogeneous 3-dimensional structure (45). The dorsal subdivision of the SCN was identified by examining every 5th 8 micron Nissl stained coronal section through the entire rostral-caudal extent of the nucleus. The center of the dorsal subdivision was then determined on the basis of cell density and overall nuclear diameter. A 200 micron region on either side of

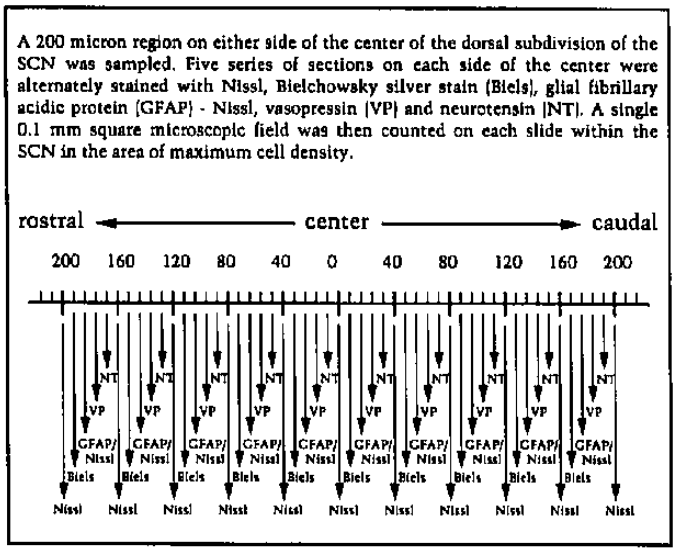

Fig. 1. Diagrammatic representation of the methodology used to sample the dorsal subdivision of the SCN.

the center of this dorsal region was sampled by staining 5 series of sections with Nissl, Bielchowsky silver, anti-GFAP antibody/ Nissl, anti-VP antibody, and anti-NT antibody (Fig. 1). A single 0.1 mm^2 field in the area of greatest cell density was then selected on each section for analysis (Fig. 2). A homogeneous distribution of both neurotensin and vasopressin neurons was consistently evident on all sections used for our study.

Morphometric Analyses

Morphometric analyses were performed with NIH Image (version 1.51). Slides were viewed with an Olympus microscope and the 10 random 0.1mm^2 fields within the dorsal SCN were digitized and analyzed with a Macintosh IIci computer. Image files were then archived to 40M removable hard disks (Syquest format, PLI turbo 40 drive) for permanent storage.

Immunocytochemistry

All of the immunocytochemical procedures were performed using the avidin-biotin complex (ABC) technique on either 40 micron free floating or 8 micron slide mounted sections in accordance with the manufacturer's specifications (Vector Elite, Burlingame, Calif.). The specific antibodies used in our studies

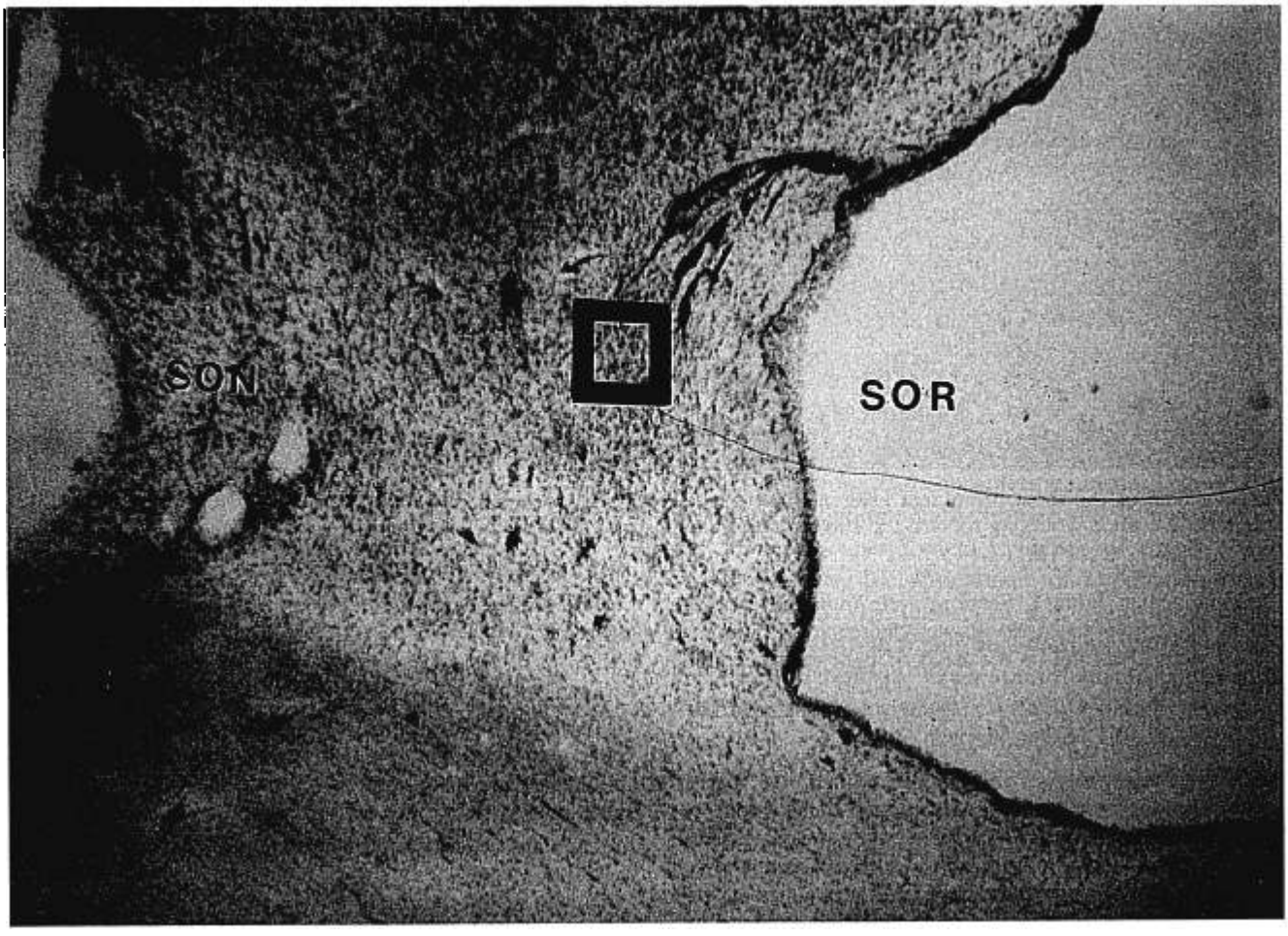

Fig. 2. The dorsal subdivision of the suprachiasmatic nucleus can be seen adjacent to the lateral border of the supraoptic recess (SOR) and medial to the supraoptic nucleus (SON) (25×). The 0.1 mm^2 field typically sampled is illustrated overlying the SCN.

TABLE 1
Antibodies Used in the Present Study

Substance	Abbreviation	Immunized species	Description of antibody	Dilution in TBS	Source
β-amyloid	β/A4	Mouse	Monoclonal	1:1,000	Boehringer
Arginine-vasopressin	VP	Rabbit	Polyclonal	1:500	Incstar
Glial fibrillary acidic protein	GFAP	Rabbit	Polyclonal	1:500	DAKO
Neuropeptide Y	NPY	Rabbit	Polyclonal	1:500	Peninsula
Neurotensin	NT	Rabbit	Polyclonal	1:500	Incstar
Vasoactive intestinal peptide	VIP	Rabbit	Polyclonal	1:500	Incstar

Fig. 3. One of the most prominent efferent fiber projections arising from the human SCN consists of this plexus of VIP immunoreactive fibers coursing toward the sub-paraventricular zone of the PVN (40×).

are summarized in Table 1. Sections were mounted on Fisher Code-On Plus slides to ensure tissue adherence. Immunoreactive cells were identified on the basis of their brown granular reaction product.

SCN Glia/Neuron Ratio

Glial proliferation occurs in response to neuronal loss. To determine the degree of neuronal loss within the SCN, the relative ratio of reactive astrocytes to neurons was assessed. Random GFAP/Nissl-stained sections chosen from the dorsal subdivision of the SCN were photographed at 400× and digitized for image analysis. Cells were identified by their respective astrocytic or neuronal staining patterns and counted to determine the ratio of reactive astrocytes to neurons. Only cells with complete cytoplasmic borders and a clearly visible nucleus were recorded. In each case, the counts obtained from 10 fields were averaged.

SCN Vasopressin and Neurotensin Neurons

To assess the degree of VP and NT neuronal loss within the SCN, 10 random fields in the dorsal SCN were photographed at 400× magnification, digitized for image analysis, and counted to determine the number of VP and NT neurons per 0.1mm² microscopic field, a measurement of cell density.

RESULTS

Neuropathologic Examination

Out of the 30 dementia cases in our study, 22 fulfilled the criteria for advanced Alzheimer disease (Braak and

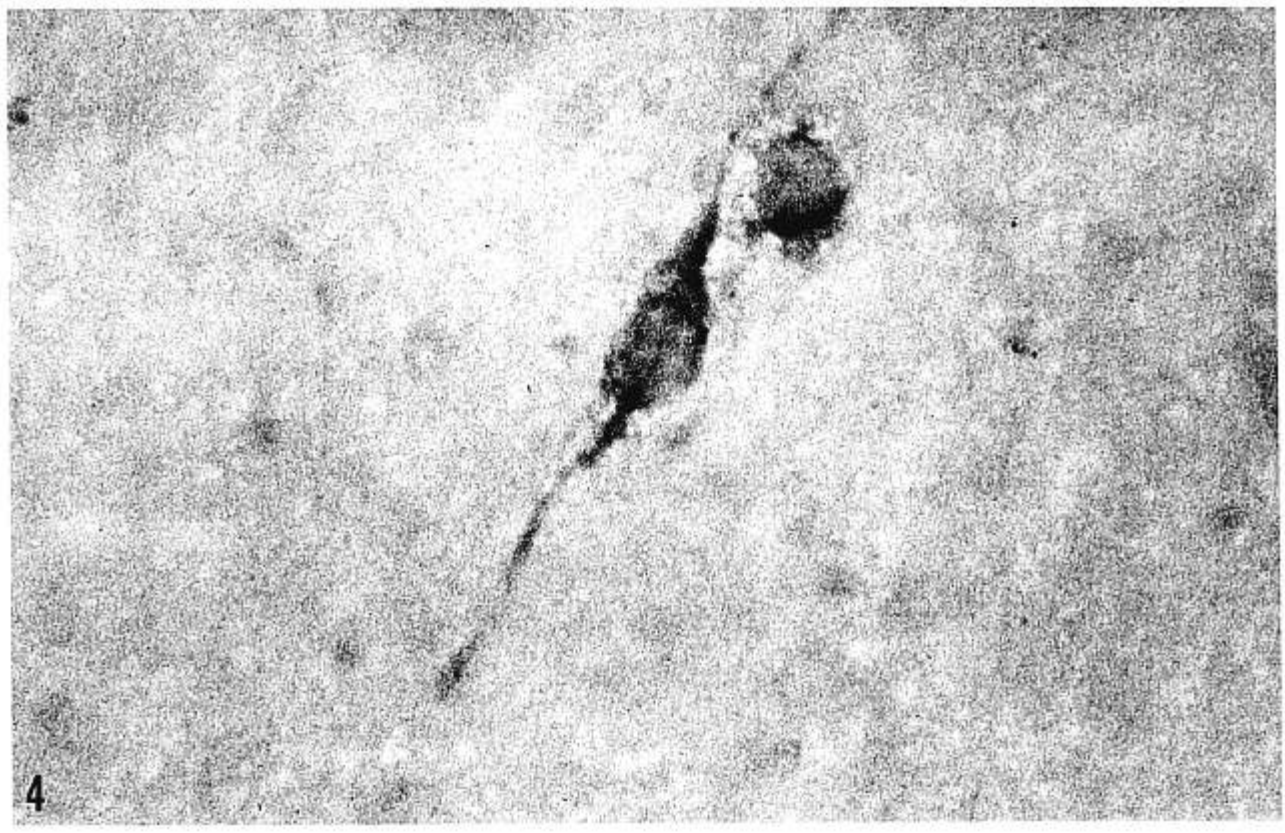

Fig. 4. NPY immunoreactive neuronal cell bodies within the human SCN (400×).

Braak stage V–VI). 3 dementia patients were found to have both advanced AD and concurrent Parkinson disease. 3 were found to have Pick disease, and 2 were found to have severe hippocampal sclerosis as well as arteriolar sclerosis. One of the patients with hippocampal sclerosis had multiple lacunes in the basal ganglia that extended into the neighboring hypothalamus, but did not involve the SCN.

Pathologic Findings within the SCN

In normal-aged controls, the size and shape of the nucleus varied with the specific staining technique(s) employed. In general, the Nissl-stained nucleus was smaller than the immunocytochemically-defined nucleus. The dorsal subdivision of the SCN was consistently recognized as the area of greatest cellularity and nuclear diameter, located medial to the supraoptic nucleus and adjacent to the lateral border of the supraoptic recess of the third ventricle. In all cases, the dorsal subdivision was found to contain abundant VP and NT neurons. A spherical shaped cluster of VIP neurons was located ventral to the VP and NT neurons. These VIP neurons were best seen on 40 micron 4% paraformaldehyde fixed sections. No other VIP neurons were evident within the preoptic area. A prominent VIP-containing fiber projection was seen coursing dorsally toward the sub-paraventricular zone of the PVN (Fig. 3). A similar efferent VIP fiber projection has been previously described in the rodent (46). Scattered NPY neurons and processes were evident, particularly within the ventral SCN (Fig. 4). As previously reported by others, the presence of NPY neuronal cell bodies is a unique feature of the human SCN (22–23).

Comparisons between AD and aged control SCN showed no evidence of complete neuronal loss of any of the 3 major peptide subdivisions or NPY neurons. There was, however, unequivocal evidence of reactive gliosis characterized by the appearance of hypertrophic gemistocytes (Fig. 5a, b). Gliosis of this nature is characteristically associated with neuronal cell loss. Sections stained with the Bielchowsky silver stain revealed scattered neuronal neurofibrillary tangles with a frequency that ranged from 0–3/section (Fig. 6). Anti-B/A4, thioflavin S stains and Bielchowsky silver stains for diffuse and mature neuritic plaques were generally unremarkable within the SCN except for rare diffuse type plaques (0–5/SCN). This finding was somewhat surprising, since abundant diffuse type plaques were more readily apparent in the adjacent hypothalamus and basal ganglia (Fig. 6). The plaques observed appeared to be similar to the hypothalamic plaques previously described by others in advanced

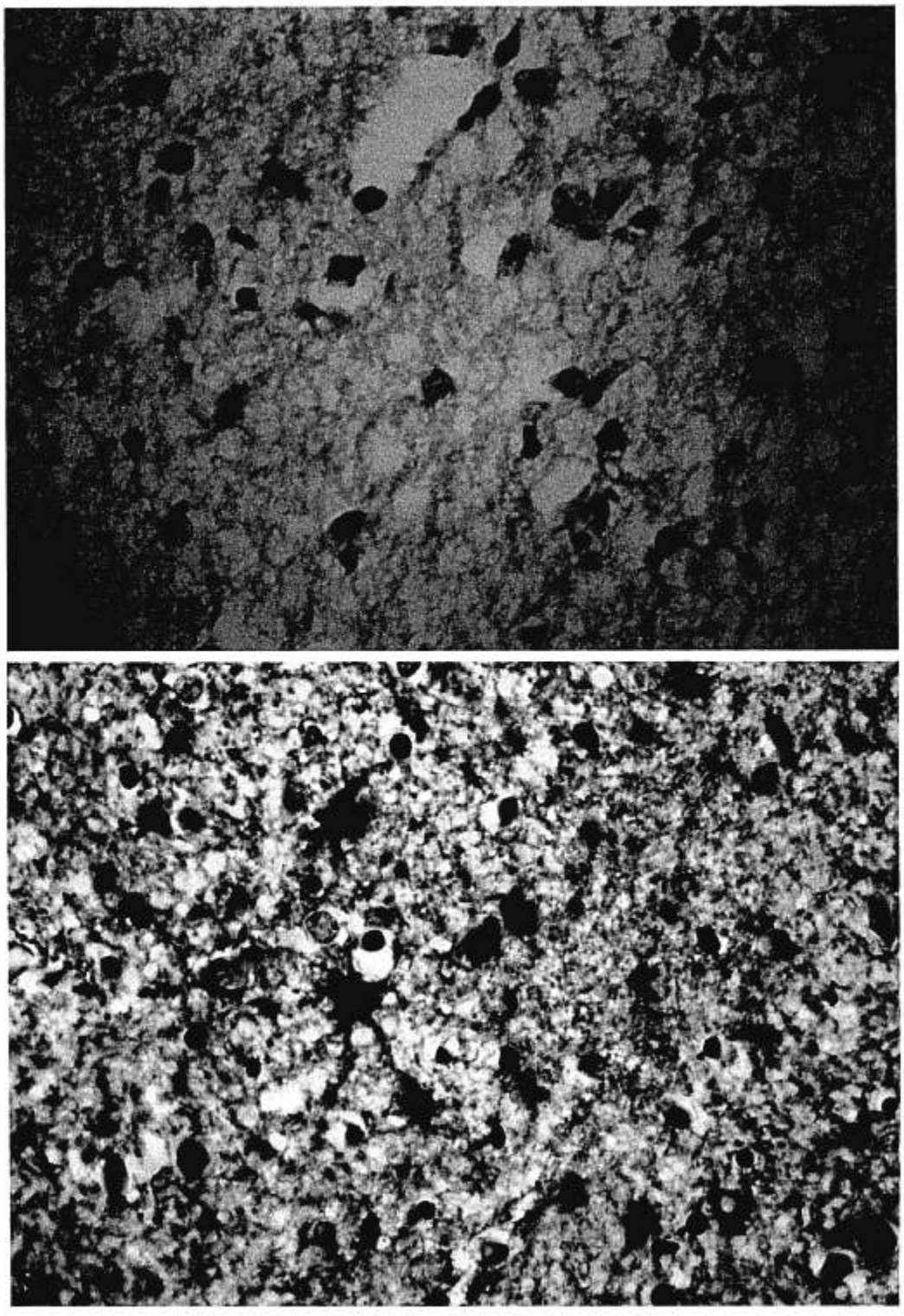

Fig. 5. High power photomicrographs of the dorsal SCN stained with anti-GFAP antibody to highlight astrocytes and counterstained with Nissl to demonstrate neurons (400×). A typical control case is shown in **a.** A case of end-stage Alzheimer Disease is shown in **b.** Numerous hypertrophic GFAP positive gemistocytic astrocytes are clearly seen in Alzheimer Disease and are notably absent in the age matched control patient.

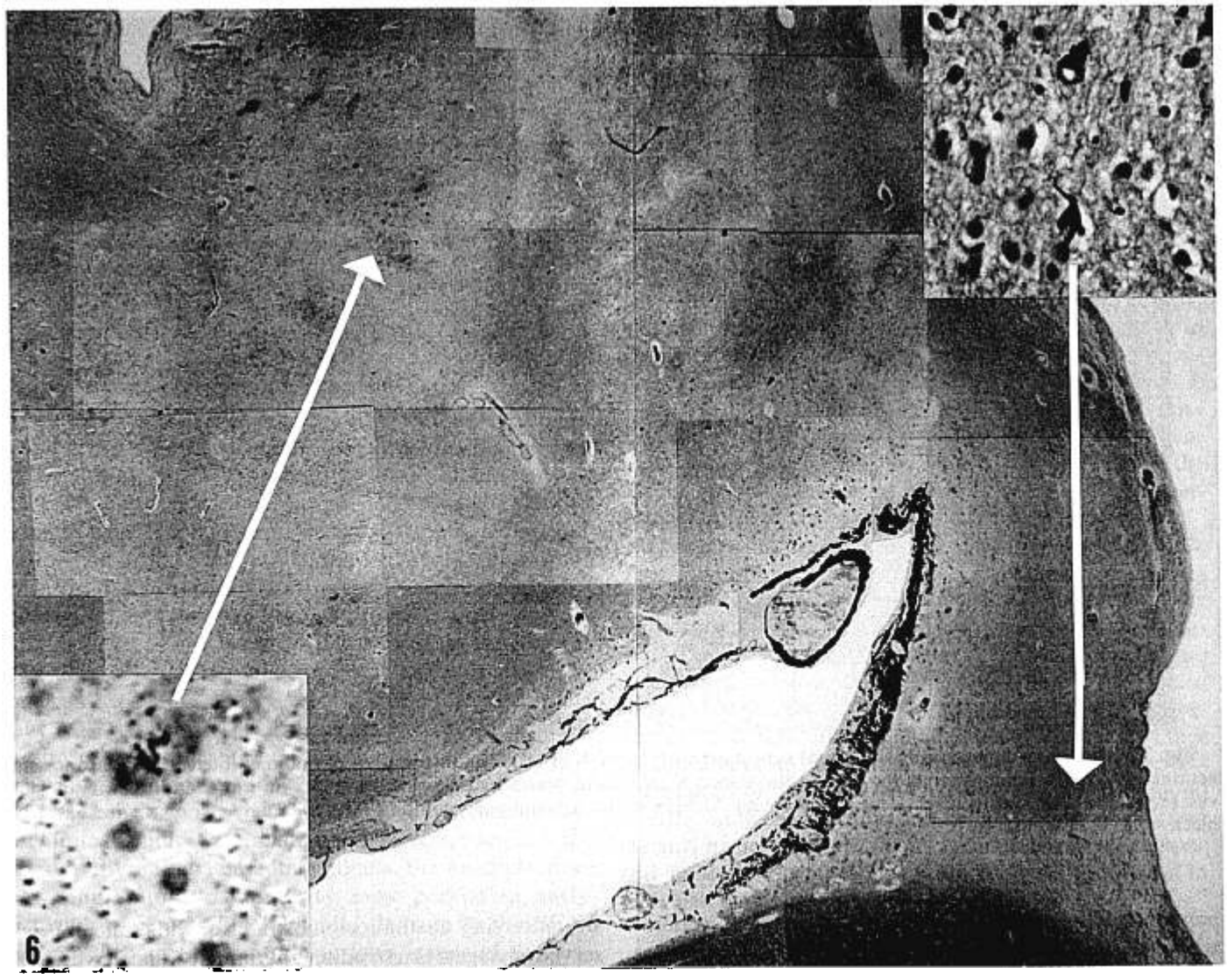

Fig. 6. Composite montage reconstructed from multiple 100× microscopic fields demonstrating the pathologic findings in a representative case of advanced of Alzheimer Disease. Senile plaques were routinely noted within the basal ganglia (lower inset) together with abundant neurofibrillary tangles in the basal forebrain (not shown). Within the hypothalamus, rare neurofibrillary tangles were noted in the SCN (upper inset). These averaged approximately 0–3/section. In general, senile plaques were extremely rare (0–5/SCN), even in our cohort of cases with advanced disease. Only diffuse-type plaques were noted within the SCN.

Alzheimer disease (47). No mature neuritic plaques were observed within the SCN.

Morphometric Analyses

Comprehensive morphometric analyses of the SCN are difficult to perform because of a variety of factors including: inter-subject variability in brain size and shape, poor demarcation of the caudal SCN boundaries, and potential variation in SCN size due to changing physiological and seasonal parameters. In view of these factors, we chose a stereological approach aimed at sampling the dorsal subdivision of the SCN. This region is composed primarily of vasopressin and neurotensin neurons (Fig. 7), and is large and easily identified by an experienced observer. Great care was taken to insure the homogeneity of the area sampled in each case studied.

A summary of the data obtained from our morphometric and neuropathological analyses is shown in Table 2. These data indicate a significant increase in the astrocyte/neuron ratio and a corresponding decrease in the number of both vasopressin and neurotensin neurons/ $0.1mm^2$ in patients with pathological evidence of Alzheimer disease, as well as those with both Alzheimer disease and Parkinson disease. They also support the conclusion that a similar trend toward SCN degeneration can be seen in patients with Pick disease, which has not been

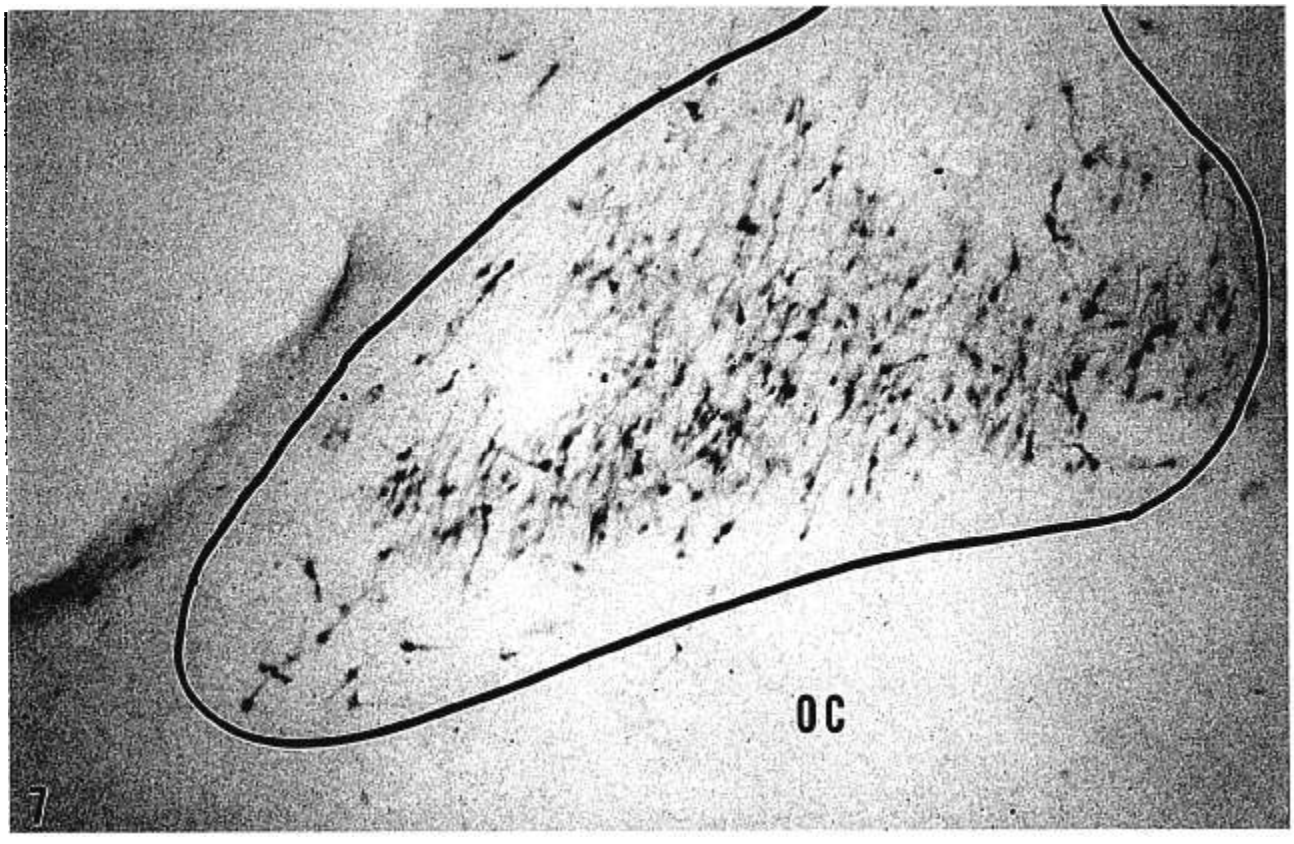

Fig. 7. Neurotensin neurons in the dorsal subdivision of the SCN (10×). The distribution of both neurotensin and vasopressin neurons was fairly homogeneous throughout the dorsal SCN. There was no significant difference in the overall distribution of vasopressin or neurotensin neurons in patients with Alzheimer disease and aged controls. The SCN boundaries are indicated in black. (OC = optic chiasm).

previously studied. Of special interest are the 2 patients who presented clinically with dementia, but who were subsequently found to have evidence of hippocampal sclerosis. One of these patients had hypertensive changes with multiple lacunar infarcts and a corresponding increased astrocyte/neuron ratio within the SCN. The other had underlying hypertensive changes, but no evidence of lacunar pathology, and a normal SCN astrocyte/neuron ratio. No senile plaques or neurofibrillary tangles were found in either of these 2 cases.

DISCUSSION

Pathologic alterations in the human hypothalamus have been noted in patients with Alzheimer Disease (47–51), although few studies have focused specifically on the SCN (21). Our findings indicate that pathologic alteration within the SCN of AD dementia patients is primarily characterized by neuronal loss and neurofibrillary tangle formation. In view of the disease severity throughout the brain (Braak and Braak stage V–VI), the damage observed within the SCN was generally less severe than that seen in other brain regions. Taken collectively, our data support the hypothesis that damage to the SCN may be an underlying anatomical substrate for the clinically observed changes in circadian rhythmicity that have been observed in many dementia patients, regardless of the underlying pathologic process.

A previous morphometric study of the SCN in aged control and AD patients demonstrated significant decreases in total SCN cell numbers and in the total number of vasopressin-containing neurons in healthy elderly, which were even more pronounced in Alzheimer Disease patients (21). Vasopressin and total SCN cell densities were not found to be significantly reduced, although both of these parameters were lowest in the dementia patients. Studies of the human SCN in Alzheimer disease by other investigators, however, have concluded that there was no evidence of significant neuronal loss or alteration of normal SCN anatomy (24, 52).

In contrast to previous studies, the present investigation was specifically limited to patients who fulfilled both clinical and pathological criteria (Braak and Braak stage V–VI) for advanced dementia. Our findings support the conclusion that there is neuronal loss within the SCN of advanced AD patients, and indicate that this neuronal loss

will be reflected by a significant decrease in vasopressin neuronal cell density.

One possible explanation for the reported inconsistencies is that the SCN is not significantly affected until the advanced stages of dementia. It would therefore be possible to have a group of patients who readily fulfill the minimum pathologic criteria for the diagnosis of Alzheimer disease, but who do not manifest significant changes within the SCN relative to age matched controls.

Another potential variable could be the different techniques utilized for the morphometric analyses as well as those used to define the anatomical boundaries of the SCN. Previous studies have focused on neuronal cell counts or cell densities. It is quite possible that the loss of specific neuropeptide markers does not always correspond to the loss of neurons. With longstanding, slowly progressive degeneration, neuronal loss is also often difficult to detect because of collapse and remodeling of the parenchyma with attendant reestablishment of normal neuronal densities. Studies have shown that in some brain areas in Alzheimer patients, cortical thickness actually increases during the disease (53). In view of this possibility, the gliosis that accompanies neuronal degeneration may be a more sensitive indicator of cell loss than neuronal cell counts or densities. Our data indicate that advanced Alzheimer disease is associated with an increase in gliosis within the SCN.

The apparent discrepancies in pathologic data may also explain the variability in conclusions reached during clinical studies of circadian parameters, such as temperature rhythm, in dementia patients. For example, normal temperature rhythmicity may persist in early dementia patients with only mild damage to the SCN, but may become altered during later stages of the disease (11). This hypothesis is supported by the observation in the rodent model that the circadian temperature rhythm persists to varying degrees with only partial lesions of the SCN (54).

Given the widespread severity of brain damage in patients with advanced dementia, it is quite likely that degeneration elsewhere in the central nervous system may cause or significantly contribute to the alteration of circadian rhythmicity in Alzheimer disease. For example, widespread axonal degeneration in the optic nerves of AD patients has been reported with an associated loss of ganglion cells in the retina (55). As previously noted, a retinohypothalamic tract within the optic nerve has been demonstrated in human postmortem tissue (28–30) that may provide the anatomic substrate for the finding that bright light is a potent entraining stimulus for human circadian rhythms (56). It is presently unknown to what extent damage to the RHT or other afferent visual pathway, the geniculohypothalamic tract (GHT), may contribute to the alteration of human circadian rhythmicity in Alzheimer disease. Another possible factor in AD patients that could interfere with normal circadian rhythmicity is loss of cholinergic function (57). Studies in experimental animals indicate that cholinergic agonists can mimic the effects of light on the entrainment of circadian wheel-running activity and pineal gland activity (58, 59).

The absence of either diffuse or mature neuritic plaques within the SCN of patients with severe dementia was an unexpected finding, especially in view of their abundance in the adjacent hypothalamus and basal ganglia. The reason for this apparent resistance to plaque formation is unclear, although other regions of the brain, such as the cerebellum, show resistance to plaque formation. The few diffuse plaques observed were similar in appearance to those described previously in the hypothalamus (47). These hypothalamic plaques were found to differ in their molecular and cellular composition from the mature neuritic plaques present in the hippocampus and neocortex, suggesting a fundamental difference in their pathogenesis.

In summary, our data indicate that the SCN is clearly affected by the pathologic alterations seen in Alzheimer disease and other forms of dementia, but appears to be less severely affected than other brain regions. Additional studies are necessary to correlate clinical symptomatology with brain pathology before firm conclusions regarding the role of the SCN in the pathogenesis of the circadian abnormalities that occur in Alzheimer disease can be decided.

TABLE 2
SCN Pathology by Diagnosis in 27 Dementia Patients

Diagnosis	n	Astrocyte/ Neuron ratio/ 0.1 mm²	p value v. control	Vasopressin Neurons/ 0.1 mm²	p value v. control	Neurotensin Nuerons/ 0.1mm²	p value v. control
AD	19	0.54 ± 0.49	0.009	9.75 ± 2.36	<0.001	6.82 ± 2.16	0.002
AD and PD	3	0.62 ± 0.24	<0.001	8.30 ± 1.05	0.09	6.37 ± 0.56	0.011
Pick disease	3	0.48 ± 0.25	0.002	8.07 ± 3.32	0.09	6.75 ± 1.49	0.037
HS*	2	0.05, 0.67	—	12, 6.7	—	7.8, 5.7	—
Controls	10	0.10 ± 0.10		16.7 ± 7.55		9.63 ± 1.77	

Data expressed as mean ± standard deviation. Statistical analyses were accomplished by the Student *t* test.
* HS = hippocampal sclerosis, numbers are individual values. ×1, ×2.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Elliott Albers, Dr. Charles Marotta, Monica Rodriguez-Wolf, Philip Song, and Michael Lebow for their help and suggestions. We also thank Robin Kiernan and Mark Morgenstern for their assistance in the preparation of this manuscript.

This paper is dedicated to the late Dr. Ronald Majocha. His advice and support during this investigation were greatly appreciated. He will be deeply missed.

REFERENCES

1. Reisberg B, Borenstein J, Salob SP, Ferris SH, Franssen E, Georgotas A. Behavioral symptoms in Alzheimer's disease: phenomenology and treatment. J Clin Psychiatry 1987;48(5 suppl):9–15
2. Evans LK. Sundown syndrome in institutionalized elderly. J Am Geriatr Soc 1987;35:101–8
3. Czeisler CA, Dumont M, Duffy JF, et al. Association of sleep-wake habits in older people with changes in output of circadian pacemaker. Lancet 1992;340:933–36
4. Vitiello MV, Smallwood RG, Avery DH, Pascualy RA, Martin DC, Prinz PN. Circadian temperature rhythms in young adult and aged men. Neurobiol Aging 1986;7:97–100
5. Weitzman ED, Moline ML, Czeisler CA, Zimmerman JC. (1982). Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. Neurobiol Aging 1982;3:299–309
6. Czeisler CA, Allan JS, Strogatz SH., et al. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science 1986;233:667–71
7. Okawa M, Mishima K, Hishikawa Y, Hozumi S, Hori H, Takahashi K. Circadian rhythm disorders in sleep-waking and body temperature in elderly patients with dementia and their treatment. Sleep 1991;14:478–85
8. Prinz PN, Christie C, Smallwood R, Vitaliano P, Bokan J, Vitiello MV, Martin D. Circadian temperature variation in healthy aged and in Alzheimer's disease. J Gerontol 1984;39:30–35
9. Prinz PN, Moe KE, Vitiello MV, Marks AL, Larsen LH. Entrained body temperature rhythms are similar in mild Alzheimer's disease, geriatric onset depression and normal aging. J Geriatr Psychiatry Neurol 1992;5:65–71
10. Touitou Y, Reinberg A, Bogdan A, Auzeby A, Beck H, Touitou C. Age-related changes in both circadian and seasonal rhythms of rectal temperature, with special reference to senile dementia of Alzheimer type. Gerontol 1986;32:110–18
11. Satlin A, Volicer L, Stopa EG, Harper D. Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease. Neurobiol Aging 1995;16:765–71
12. Rusak B, Zucker I. Neural regulation of circadian rhythms. Physiol Rev 1979;59:449–526
13. Moore RY. The suprachiasmatic nucleus and the organization of a circadian system. Trends Neurosci 1982;5:404–7
14. Von Economo C. Die Encephalitic Lethargica, Urban and Schwarzenberg, Wien, 1929
15. Fulton JT, Bailey. Tumors in the region of the 3rd ventricle: Their diagnosis and relation to pathological sleep. J Nerv Ment Dis 1929;69:1–25, 145–64, 261–77
16. Schwartz WJ, Busis NA, Hedley-Whyte ET. A discrete lesion of ventral hypothalamus and optic chiasm that disturbed the daily temperature rhythm. J Neurol 1986;233:1–4
17. Le Gros Clark WE. In: Le Gros Clark WE, Beattie J, Riddock G, Dott N, eds. The hypothalamus: Morphologic, functional, clinical and surgical aspects. Oliver and Boyd: Edinburgh, 1938:1–68
18. Defendini R, Zimmerman EG. In: Reichlin S, Baldessarini RJ, Martin JB, ed. The hypothalamus. Raven Press: New York,1978, pp. 137–52
19. Sofroniew MV, Weindl A, Schell Y, Wetzstein J. Immunohistochemistry of vasopressin, oxytocin and neurophysin in hypothalamus and extrahypothalamic regions of human and primate brain. J Acta Histochemica (Suppl) 1981;24:79–95
20. Stopa EG, King JC, Lydic R, Schoene WC. (1984). Human brain contains vasopressin and vasoactive intestinal peptide neuronal subpopulations in the suprachiasmatic region. Brain Res 1984;297: 159–63
21. Swaab DF, Fliers E, Partman TS. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res 1985;342:37–44
22. Mai JK, Kedziora O, Teckhaus L, Sofroniew MV. Evidence for subdivision in the human suprachiasmatic nucleus. Comp Neurol 1991;305:508–25
23. Moore RY. Organization of the primate circadian system. J Biol Rhythms 1993;(8 Suppl.):53–59
24. Gao B, Moore RY. Glutamic acid decarboxylase message isoforms in human suprachiasmatic nucleus. J Biol Rhythms 1996;11:172–9
25. Hofman MA, Zhou JN, Swaab DF. Suprachiasmatic nucleus of the human brain: an immunocytochemical and morphometric analysis. Anat Rec 1996;244:552–62
26. Mai JK, Triepel J, Metz J. Neurotensin in the human brain. Neurosci 1987;22:499–524
27. Reppert SM, Weaver DR, Rivkees SA, Stopa EG. Putative melatonin receptors in a human biological clock. Science 1988;242: 78–81
28. Sadun AA, Schaechter JD, Smith TH. A retino-hypothalamic pathway in man: Light mediation of circadian rhythms. Brain Res 1984;302:371–77
29. Friedman DI, Johnson JK, Chorsky RL, Stopa EG. Labeling of the human retino-hypothalamic tract with the carbocyanine dye, DiI. Brain Res 1991;560:297–302
30. Moore RY, Speh JC. A putative retionhypothalamic projection containing substance-P in the human. Brain Res 1994;659:249–53
31. Hofman MA, Fliers E, Goudsmit E, Swaab DF. Morphometric analysis of the suprachiasmatic and paraventricular nuclei in the human brain: Sex differences and age-dependent changes. J Anatomy 1988;160:127–43
32. Hofman MA, Swaab DF. Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res 1994;651:134–42
33. Hofman MA, Swaab DF. Diurnal and seasonal rhythms of neuronal activity in the suprachiasmatic nucleus of humans. J Biol Rhythms 1993;8:283–95
34. Swaab DF, Goudsmit E, Kremer HP, Hofman MA, Ravid R. The human hypothalamus in development, sexual differentiation, aging and Alzheimer's disease. Prog in Brain Res 1992;91:465–72
35. Hofman MA, Purba JS, Swaab DF. Annual variations in the vasopressin neuron population of the human suprachiasmatic nucleus. Neuroscience 1993;53:1103–12
36. Hofman MA, Swaab DF. Seasonal changes in the suprachiasmatic nucleus of man. Neuroscience Letters 1992;139:257–60
37. Swaab DF, Roozendaal B, Ravid R, Velis DN, Gooren L, Williams RS. Suprachiasmatic nucleus in aging, Alzheimer's disease. Progress in Brain Res 1987;72:301–10
38. Swaab DF, Hofman MA. An enlarged suprachiasmatic nucleus in homosexual men. Brain Res 1990;537:141–48
39. Hofman MA, Zhou JN, Swaab DF. No evidence for a diurnal vasoactive intestinal polypeptide (VIP) rhythm in the human suprachiasmatic nucleus. Brain Res 1996;722:78–82
40. Zhou JN, Hofman MA, Swaab DF. VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. Neurobiol Aging 1995;16:571–76
41. Zhou JN, Hofman MA, Swaab DF. No changes in the number of vasoactive intestinal polypeptide (VIP)-expressing neurons in the

suprachiasmatic nucleus of homosexual men; comparison with vasopressin-expressing neurons. Brain Res 1996;72:285–88
42. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44
43. Khachaturian ZS. Diagnosis of Alzheimer's disease. Arch Neurol 1985;42:1097–105
44. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 1991;82:239–59
45. Weibel ER. Sampling of tissue. In: Stereological Methods, Volume 1. New York: Academic Press, 1989:Chapter 3:63–100
46. Watts AG. The efferent projections of the suprachiasmatic nucleus: Anatomical insights into the control of circardian rhythms. In: Klein DC, Moore RY, Reppert SM, eds. Suprachiasmatic nucleus: The minds clock. New York: Oxford University Press, 1991:77–106
47. Standaert DG, Lee VM, Greenberg BD, Lowery DE, Trojanowski JQ. Molecular features of hypothalamic plaques in Alzheimer's disease. Am J Pathol 1991;139:681–91
48. Ishi T. Distribution of Alzheimer's neurofibrillary changes in the brain stem and hypothalamus of senile dementia. Acta Neuropathol 1966;16:181–87
49. Swaab DF, Grundke-Iqbal I, Iqbal K, Kremer HO, Ravid R, van de Nes JA. Tau and ubiquitin in the human hypothalamus in aging and Alzheimer's disease. Brain Res 1992;590:239–49
50. Saper CB, German DC. Hypothalamic pathology in Alzheimer's disease. Neuroscience Letters 1987;74:364–70
51. Schultz C, Ghebremedhin E, Braak H, Braak E. Neurofibrillary pathology in the human paraventricular and supraoptic nuclei. Acta Neuropathol (Berl) 1997;94:99–102
52. Moore RY, Speh JC, Suhan N. The suprachrasmatic nucleus in Alzheimer's disease [abstract]. Society for Neuroscience 1994;71.1:158
53. Terry RD, Peck A, DeTeresa R, Schechter R, and Horoupian DS. Some morphometric aspects of the brain in senile dementia of the Alzheimer's type. Ann Neurol 1981,10:184–92
54. Kittrell EMW. The suprachiasmatic nucleus and temperature rhythms. In: Klein DC, Moore RY, Reppert SM, eds. Suprachiasmatic nucleus: The minds clock. New York: Oxford University Press, 1991:233–45
55. Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic nerve degeneration in Alzheimer's disease. N Eng J Med 1986;315:485–87
55. Czeisler CA, Allan JS, Strogatz SH, et al. Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science 1986;233:667–71
57. Davies P, Maconey AJF. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 1976;2:1403
58. Zatz M, Brownstein MJ. Injection of alpha-bungarotoxin near the suprachiasmatic nucleus blocks the effect of light on nocturnal pineal enzyme activity. Brain Res 1981;213:438–42
59. Zatz M, Herkenham MA. Intraventricular carbachol mimics the phase shifting effect of light on the circadian rhythm of wheel-running activity. Brain Res 1981;212:234–38.

Received April 6, 1998
Revision received August 13, 1998
Accepted September 21, 1998