Circadian system rhythm disorders in aging and Alzheimer’s disease. Role of changes in melatonin, suprachiasmatic nucleus and corticosteroids

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Circadian rhythms are a fundamental feature of all living systems. Different biological phenomena such as rest-activity, sleep-wakefulness, body temperature and cognitive performance all exhibit a profound 'circadian' rhythmicity, i.e., they reveal a free running period of about 24 hours. The involvement of these rhythms in physiology and pathology have been the subject of many studies. Spontaneous births are most prevalent at night (Swaab et al. 1996), myocardial infarcts and strokes occur more often in the morning (Lemmer, 1997). Between these two life events, birth and death, our behavioral and metabolic functions change progressively and predictably over the 24 h cycle. The internal temporal program allows us to anticipate the day-night rhythms in the outside world and is therefore an important adaptive mechanism. Disruption of the program, as seen for example in shift workers, can carry a severe penalty with poor mental and physical performance and diminished sense of well-being (Smith et al. 1994). The aging clock shows a progressive loss of precision and sensitivity to light (Zhang et al. 1996) which might underlie the poor quality of sleep that is such a chronic and characteristic problem for the growing population of elderly people (Brock, 1991). A better understanding of circadian neurobiology may not only help us improve the quality of life of such people significantly but may also allow us to explain how complex brain functions and behavior can arise from the intrinsic properties of a relatively small number of cells, i.e. 50,000, (Swaab, 1995) of a well defined chemical nature (Swaab, 1999). In human beings, the circadian timing system consists of two major brain structures: the suprachiasmatic nucleus (SCN) and the pineal gland. The SCN, containing the endogenous clock of the brain, generates and regulates endogenous circadian rhythms and induces circadian changes in various brain regions by virtue of its innervation (Dai et al. 1998b; Dai et al. 1998a; Dai et al. 1998c). The SCN also innervates the pineal gland by a complex, polysynaptic pathway (Moore & Klein, 1974; Klein, 1978; Foulkes et al. 1997; Cassone, 1990; Kalsbeek et al. 1999). Melatonin, the hormone of the pineal, will transfer the time information to other parts of the brain and body (Figure 1). In a similar way also other endocrine glands, such as adrenals and thyroid gland, may serve to amplify the rhythms generated in the SCN.
Fig. 1 Diagram of the human brain (mid-sagittal section) showing the neural pathways (dashed line) by which photoperiodic information reaches the pineal. Abbreviations: SCN, suprachiasmatic nucleus; PVN, paraventricular nucleus; SCG, superior cervical ganglion. From Hofman and Swaab, 1992.
I Suprachiasmatic nucleus: biological clock of the brain, functions and chemoarchitecture

The SCN consists of a paired nucleus of about 0.25 mm³ (Swaab et al. 1985b) situated just above the optic chiasm. It is the major and probably only circadian pacemaker of the mammalian brain and coordinates hormonal and behavioral circadian rhythms (Rusak & Zucker, 1979). In a process called entraining, this endogenous SCN rhythm is synchronized to the 24-hour environmental light-dark cycle. Entraining occurs through a direct neuronal pathway from the retina to the SCN. This was not only shown by staining degenerating fibers in patients with optic nerve damage (Sadun et al. 1983), but also by a recently developed postmortem tracing technique. The retinohypothalamic tract leaves the optic chiasm and innervates mainly the ventral part of the SCN directly (Dai et al. 1998b; Dai et al. 1998a; Dai et al. 1998c). The retinohypothalamic tract is the principal pathway mediating the entraining effects of light on the SCN. A few clinical observations support the importance of the SCN for circadian rhythms in human. A lesion in the suprachiasmatic region of the human hypothalamus, e.g., as the result of a tumor, indeed results in disturbed circadian rhythms in human beings (Cohen & Albers, 1991; Haugh & Markesbery, 1983). Moore (1992) briefly reported on a patient with an optic nerve glioma who had evidence of loss of rhythmicity in several functions and compression of the chiasmatic area visualized by a CT scan (Moore, 1992). Persons who are completely blind because of a damaged retina or optic tract often lack the entraining effects of light and may show free-running temperature, cortisol and melatonin rhythms. They may also suffer from sleep disturbances (Lewy & Sack, 1996; Sack et al. 1992). However, patients who are blind because of damage to the visual cortex may still entrain their circadian rhythm (Czeisler et al. 1995), which emphasizes the importance of the light-dark cycle for synchronizing the circadian activity of the SCN.

The hypothesis that the SCN is the biological clock of the brain has been confirmed in several animal models. Two groups showed independently that bilateral lesion of a small area in the ventral hypothalamus, just dorsal to the optic chiasm, disrupted the normally occurring daily fluctuation in the adrenal corticosterone content (Moore & Eichler, 1972) and that of locomotor activity and drinking behavior (Stephan & Zucker, 1972). Further studies revealed that complete destruction of the SCN abolished all daily rhythms, hormonal as well as behavioral. In contrast, in rats, circadian rhythmicity is maintained when different parts of the optic system (e.g., the optic cortex) are lesioned. Only after enucleation of both eyes do the rhythms become free running, and a lesion directly in the SCN itself causes complete arrhythmicity (Stephan & Zucker, 1972; Moore & Eichler, 1972). Conclusive evidence that the SCN contains the major circadian pacemaker in the mammalian central nervous system was provided by the elegant experiment of Ralph et al. (1990). They showed that transplantation of the fetal SCN area into the hypothalamus of an SCN-lesioned animal resulted in restoration of the circadian activity pattern...
in such a way that the host animal adapted to the circadian rhythm of the SCN of the donor. Transplantation studies were performed using the tau mutant hamster, of which the heterozygote form has a circadian period length of about 22 h and the homozygote has a period of about 20 h. Donor SCN obtained from a homozygous tau mutant hamster re-instated a 20 h rhythm in an arrhythmic SCN lesioned host animal with a 24 h rhythm prior to lesion and vice versa (Ralph et al. 1990), showing that the restored rhythm is always derived from the donor SCN. The percentage of rats recovering circadian rhythmicity was, however, relatively low (about 40%), which may be due to the limited reinnervation of the host brain by the graft (Griffioen et al. 1993; Sollars et al. 1995; Wiegand & Gash, 1988). We used an adenoviral vector-mediated gene transfer method with neurotrophins to try to improve neuritic outgrowth of the grafts towards denervated SCN target sites in SCN-lesioned rats in order to obtain a better restoration of the host circadian rhythmicity (Boer et al. 1997; Van Esseveldt et al. 1997).

The results from human and animal experiments indicate that there is a close structure-function relationship for circadian rhythms in the SCN. When SCN tissue is isolated in vitro, neuronal firing and vasopressin release maintain their circadian rhythmicity (Shirakawa et al. 2000). A marked diurnal and circannual oscillation was observed in the number of vasopressin-expressing neurons in the SCN of young subjects, i.e. low vasopressin neuron numbers during the night and peak values during the early morning, and low vasopressin neuron numbers during the summer, and peak values in autumn (Hofman & Swaab, 1994; Hofman & Swaab, 1993). Several animal studies reported, moreover, a relation between the number of AVPergic neurons in the SCN and the strength and consistency of circadian activity rhythms. In different rat strains and mice selected for differences in nest-building behavior, the animals with the highest number of AVPergic neurons in the SCN had the strongest unimodal activity pattern, whereas the animals with the lowest numbers of VPergic neurons had the weakest multimodal activity pattern (Bult et al. 1993; Bult et al. 1992; Wollnik & Bihler, 1996). However, the reverse was reported for common voles, where the animals with the highest number of VPergic neurons did not have circadian activity rhythms (Gerkema et al. 1994). Since rat strains with the weakest rhythm also had the highest amount of VPergic fibers in the SCN, this discrepancy was speculated to result from a reduced AVP release, rather than from an increased AVP synthesis (Gerkema et al. 1994). Indeed, recent observations (Zhou et al, 2000, submitted) have shown that, in depressed patients, normal diurnal rhythms in the number of AVP neurons in the SCN disappear, the number of neurons expressing AVP peptide increases, and the amount of AVP mRNA decreases. This indicates a decreased synthetic activity of the AVP neurons of the SCN in depressed patients and an even more decreased transport of vasopressin. It may be important to study synthesis and release in relation to differences in the strength of the rhythms.

An important feature of SCN organization is that it is a multioscillatory system where
the entire clockwork resides in single neurons (Welsh et al. 1995). Interactions among these “clock cells” in the whole SCN serve to synchronize individual circadian clocks to generate coordinated circadian output. This output ultimately controls a vast array of circadian rhythms in physiology and behavior. The presence of a genetic basis of the circadian period is supported by the phenotypic analyses of single gene mutations in organisms, from cyanobacteria to mammals, that show dramatic alterations in period. The identification of genes which appear to be crucial for circadian rhythmicity (‘clock genes’) in the fruit fly Drosophila melanogaster and the fungus Neurospora crassa, has prompted many investigators to search for mammalian homologies (Sangoram et al. 1998; Sun et al. 1997; Tei et al. 1997). The result indicated that also in mammals a similar mechanism underlies the circadian clock. In the mean time, mutagenesis studies in mouse resulted in the identification of a clock gene (Vitaterna et al. 1994) that vice versa has its homology in the Drosophila pacemaker system, and has a similar function. Finally, behavioral screens have resulted in the recognition of individual variations in the rhythmic parameters in several species. A variation was observed, for instance, in the strength of activity rhythms in a population of common voles and in different mice and rat strains (Bult et al. 1993; Bult et al. 1992; Wollnik & Bihler, 1996; Gerkema et al. 1994), where deviating rhythmic parameters were also observed in naturally occurring mutants, such as the obese Zucker rat (Murakami et al. 1995), the VP-deficient Brattleboro rat (Brown & Nunez, 1989), and the tau mutant hamster (Ralph & Menaker, 1988). Moreover, changes in circadian activity were observed in transgenic animals, such as the hyperactive Wocko mouse (Sollars et al. 1996), the prion protein knockout mouse (Tobler et al. 1996), the C-fos-deficient mice (Honrado et al. 1996) or Cry1 and Cry2 single and double mutant mouse (Van der Horst et al. 1999).

A relatively large number of neuropeptides has been identified in the human SCN. Neurons that are immunoreactive for AVP, vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY), neuropeptide-YY (NPY), neurotensin (NT) and somatostatin (SOM) are present in the SCN in a characteristic anatomical orientation (Swaab et al. 1985b; Moore, 1992; Mai et al. 1991; Stopa et al. 1984). Light and electron microscopic immunocytochemistry using antibodies against neuropeptides revealed that VIP neurons and VP neurons are major components of the SCN. The region of the SCN that receives retinohypothalamic tract input and is therefore considered to be of importance for entrainment, is characterized by VIP neurons (Moore, 1992; Stopa et al. 1984). AVP is found in the remainder of the SCN. AVP may amplify the rhythm in this nucleus by its excitatory effect during the light phase as shown by animal experiments (Ingram et al. 1996), while AVP has been shown to play a role in synchronizing circadian rhythms to the light entrainable oscillator (Murphy et al. 1998). The observation that intranasal vasopressin markedly enhanced nocturnal slow-wave sleep in human (Perras et al. 1996) should perhaps be considered in the light of the function of this neuropeptide in the SCN. A recently developed im-
munofluorescence technique showed the cellular colocalization of VIP and VP in some neurons of the SCN of the human hypothalamus (Romijn et al. 1999). Most neurons in the SCN are GABAergic (γ-aminobutyric acid) because they show glutamic acid decarboxylase (GAD) immunoreactivity and express GAD mRNA (Okamura et al. 1990). Subsequent studies have shown that one or more peptides are co-localized in most, if not all, SCN neurons with the inhibitory neurotransmitter GABA (Gao et al. 1995; Buijs et al. 1998). During the light period, when the SCN neurons are most active (Inouye & Kawamura, 1979), a large portion of the peptides is secreted, whereas during the dark period the secretion of GABA prevails. The high level of AVP released by the SCN during the (subjective) light period (Earnest & Sladek, 1986) seems to support this idea. This suggests that the function of a single SCN neuron containing AVP and GABA in its terminal may change depending on its firing frequency; excitatory during the (subjective) light period, when VP is released (VP is usually found to be excitatory) and switching to inhibitory during the dark period, when GABA is released (Buijs et al. 1998). It is clear that electrophysiological studies examining post- and presynaptic actions of GABA and AVP are necessary for understanding the functional significance of this co-localization.

The wealth of intrinsic connections containing GABA suggests that within the SCN powerful mechanisms are available to shut down the activity of this nucleus (Buijs et al. 1998). In addition, as was noted by Van den Pol (1993) a system of self-inhibiting oscillators may provide the necessary stability, while it may also explain phenomena such as synchronous firing (Buijs et al. 1998). Functional studies have to some extent confirmed the role of AVP in signaling the circadian rhythm to the SCN target areas by its efferent projections, but there is relatively little evidence that VIP serves a similar role. However, as several transmitters, in particular GABA, are colocalized in the SCN neurons containing VP and VIP; the release of a particular cocktail of colocalized transmitters might be more crucial to the signalling role of the SCN efferent than just the release of AVP or VIP. SCN projections to the sPVz, the MPN, the PVN and the DMH, may allow the SCN to modulate many neuroendocrine and autonomic functions (Buijs et al. 1998). These findings illustrate that the SCN uses a highly differentiated language to transmit its circadian signal to the rest of the brain.
II Effects of melatonin: circadian rhythm, therapeutic potential and significance to human health

The pineal gland is a relatively new arrival on the endocrine scene since its chief hormonal product, melatonin, was discovered only 41 years ago (Lerner et al. 1959). Unequivocal physiological evidence of its endocrine capabilities is even more recent (Hoffman and Reiter 1965). Melatonin (N-acetyl, 5-methoxytryptamine) is synthesized in several tissues (Klein, 1978; Cassone, 1990), but rhythmic synthesis is primarily localized in the pineal gland and retina (Cassone, 1998). Serum melatonin levels are high at night and low during the day. During darkness, norepinephrine is released from the sympathetic nerve endings in the pineal gland to activate N-acetyltransferase, the enzyme which catalyzes the rate limiting step of the synthesis of serotonin and melatonin from tryptophan. It was discovered that melatonin also had marked effects on circadian rhythmicity (Armstrong, 1989). Thus, in animals, properly timed melatonin injections were shown to synchronize circadian rhythms (Redman & Armstrong, 1988). In humans as well, melatonin has been tested for its efficacy in treating circadian rhythm disturbances associated with jet lag, shift work, delayed phase sleep disorder and REM sleep behavioral disorder (Lewy & Sack, 1996; Arendt, 1997; Kunz & Kunz, 1997).

Kloeden et al. (1990) argued in favor of the existence of a centralized clock to coordinate the genetic switching of all cells that age. Furthermore, they localized this clock in the pineal gland and speculated that the circadian melatonin signal functions as the “hands” of the clock to notify all cells in the organism of the passage of time. As Kloeden et al. (1993) envisage it, the decreased amplitude and/or duration of the nocturnal melatonin peak may be part of the signal that informs all cells in the organism not only about day/night rhythms but also about the age of the organism as a whole. Reiter et al (1992) also argue that it is unlikely that the average reduction in the amplitude of the melatonin rhythm that occurs with age or the changing duration of the nocturnal melatonin surge during aging by themselves would serve as the signal bearer of age (Reiter, 1992). It was reported that pinealectomy shortened life span in rats and described a prolongation of life by 20% by nightly melatonin administration to mice (Pierpaoli & Maestroni, 1987). They considered that their results were due to a stimulation of the immune system (Maestroni et al. 1988b; Maestroni et al. 1988a) and an anti-stress action, both operating via the effects of melatonin on endogenous opiates. These experiments were not recently followed up.

Melatonin is a hormone that not only plays a major role in the regulation of circadian rhythms (Cassone et al. 1986), but also functions as part of an anti-oxidative defense system (Reiter et al. 1995). The debilitating consequences of age-related brain deterioration are widespread and extremely costly in terms of quality of life and longevity. A potential major cause of age-related destruction of neuronal tissue is the toxic free radicals that are a natural result of aerobic metabolism (Pappolla et al. 1998; Richardson et al.
1996; Markesbery, 1997; Fahn & Cohen, 1992; Coyle & Puttfarcken, 1993). Senile plaques are among the most conspicuous neuropathological changes found in the brains of elderly individuals and one of the clinical hallmarks of Alzheimer's disease. β-Amyloid 39-42 is one of the major constituents of these plaques (Murphy et al. 1992). Pappolla (1998) found a subgroup of neurofibrillary tangles (15%-25%), the other major hallmark of Alzheimer's disease, and senile plaques (50%) that showed immunoreactivity for both superoxide dismutase (CuZn- and Mn-forms) and catalase. Tangle-free neurons in both diseased and control brains showed weak to absent intracytoplasmic immunoreactivity. This finding supports the hypothesis that oxidative stress may be involved in the pathogenesis of Alzheimer's disease (Pappolla et al. 1998). It has been speculated that development of age-related neurodegenerative conditions that involve free radical destruction of cellular organelles and neurons may relate, at least in part, to the gradual loss of melatonin in advanced age (Poeggeler et al. 1993; Reiter et al. 1994). Recently it was shown that melatonin readily passes through the blood-brain barrier and enters neurons and glial cells (Menendez-Pelaez et al. 1993) where it may have strong antioxidant activity and may be an effective free-radical scavenger (Tan et al. 1993). This implies that melatonin may provide protection to the brain from the damaging actions of oxygen-based radicals. It is also obvious that melatonin's protective actions are not restricted to the central nervous system because in the experiments with the highly toxic carcinogen, safrole, hepatic DNA was potently protected by melatonin (Reiter et al. 1994). The notion that the indole readily diffuses into every cell and into every subcellular compartment is certainly consistent with the possibility that melatonin may provide protection against oxidative attacks in every cell in the organism.

Armstrong and Redman (1991) proposed that melatonin has beneficial effects in terms of aging and age-related diseases because of its association with the circadian timing. According to these investigators, the stability of the circadian system correlates with the amplitude of the melatonin cycle and, as a result, the loss of melatonin in advanced age (Reiter, 1992) will lead to a disturbance in the circadian pacemaker, which causes internal temporal dysfunction. Interestingly, in aged rats the amount of VIP mRNA in the SCN is reduced, whereas intraperitoneal administration of melatonin produced recovery of the amount of VIP mRNA in aged rats to levels similar to those found in young rats. The observations mentioned above have spawned a number of theories advancing the idea that melatonin may be an antiaging hormone (Pierpaoli et al. 1991). The beneficial effects of melatonin on human age related disorders is, however, still far from proven. In addition to its possible involvement in neurodegeneration and its well documented effect on circadian rhythms and seasonal responses, melatonin also has hypnotic and hypothermic effects (Nave et al. 1996; Hughes & Badia, 1997; Penev & Zee, 1997), influences arterial blood flow, decreases blood pressure and blunts noradrenergic activation (Reinberg et al. 1996; Cagnacci et al. 1997). The nocturnal rise in melatonin may thus
have a protective effect against cardiovascular accidents and seems to be beneficial in the treatment of essential hypertension (Cagnacci et al. 1998). Melatonin has also been used effectively in the treatment of sleep-wake disturbances in blind children and young adults (Palm et al. 1997). Considering its widespread actions, any change in the amount of melatonin the pineal gland produces and secretes is presumed to have noticeable metabolic consequences. The endogenous melatonin profile is not affected by melatonin treatment in humans, although it can shift the phase. Consequently there is no indication for a feedback inhibition of pineal melatonin by such a therapy (Matsumoto et al. 1997).
III Relationship between the SCN and melatonin in circadian timing system

The SCN receives photic information by a direct neural pathway from the retina, which exists in all mammals studied so far, including man. This retinohypothalamic tract appears to be both necessary and sufficient for the synchronization of the period and phase of circadian rhythms to the environmental light-dark cycle. Only a small number of hypothalamic nuclei are directly innervated by the SCN (Dai et al. 1998b; Dai et al. 1998a; Dai et al. 1998c), but this nucleus may impose circadian fluctuations indirectly on many more brain structures by means of melatonin from the pineal gland (Swaab, 1999). There is a multisynaptic neural pathway by which the SCN controls the diurnal synthesis and secretion of the pineal hormone melatonin (Moore & Klein, 1974; Klein, 1978; Foulkes et al. 1997; Cassone, 1990). (Figure 1). This pathway consists of a GABAergic projection from SCN to PVN (Kalsbeek et al. 1999), projections from the PVN to the intermediolateral column of the spinal cord, and subsequently to the superior cervical ganglion, which projects to many encephalic structures, including pineal gland. There, daily rhythms of norepinephrine release drive melatonin biosynthesis through the stimulation of β- and α-adrenergic receptors situated on secretory pinealocytes. Interruption of this pathway from the SCN to the pineal by brain lesion or pharmacological blockade of β-adrenergic receptors abolishes the rhythm of pineal melatonin, underlining the view that the clock within the SCN directly regulates melatonin biosynthesis in the pineal gland (Cassone, 1998). Melatonin feeds back on the SCN by inhibition of neuronal firing in the biological clock (Shibata et al. 1989) and can entrain the phase of the circadian rhythm (Cassone, 1990). The role of melatonin in circadian behavior varies among species. For instance, in hamster the hormone mediates seasonal variation in reproductive behavior (Goldman & Darrow, 1983), while in human it has a role as 'sleep-promoting' hormone (Zhdanova & Wurtman, 1997). The sleep-promoting effects of melatonin can partly be explained by its actions on the SCN, i.e. by (i) phase shifting of the circadian pacemaker, located in the SCN, and/or (ii) attenuation/antagonism of the SCN-dependent mechanism that promotes and maintains behavioral activation at particular times of the day.

Several lines of evidence support the view that the SCN is the primary site of melatonin's circadian effects in mammals. Surgical destruction of the rat SCN abolishes circadian patterns of activity (Moore, 1983) and blocks the entraining effects of melatonin (Cassone et al. 1986). These effects do not rely on rhythmicity per se since rats rendered arrhythmic in constant light (LL) are resynchronized with daily melatonin administration (Chesworth et al. 1987). The cloning and characterization of the melatonin receptors have been reported and melatonin receptors have been found in the SCN by binding analysis and in situ hybridization (Weaver & Reppert, 1996; Dubocovich et al. 1996). Melatonin receptors in the SCN provide a link for the SCN-pineal feedback circuit. This loop does not appear to involve a conventional negative feedback inhibition as is known,
e.g., for the glucocorticoid regulation (Matsumoto et al. 1997). So melatonin presumably modulates SCN function in a different way, e.g. by phase-shifting circadian rhythms and direct inhibitory effect on the electrical activity of SCN neurons. Melatonin has been reported to advance the circadian clock only at dawn and dusk (McArthur et al. 1997). These effects may be mediated at least in part by actions that involve in the SCN.
IV Interaction between the circadian timing system and glucocorticoid levels

One of the endocrine rhythms regulated by the SCN is that of the adrenal cortex (Buijs et al. 1998). The SCN and the hypothalamo-pituitary adrenal (HPA)-axis interact in various ways, in the first place by the SCN-derived vasopressinergic projection to the dorsomedial and paraventricular nucleus of the hypothalamus (Dai et al. 1998b; Dai et al. 1998a) that is responsible for the diurnal trough of adrenal activity. SCN lesions and microperfusion of AVP in rats showed a pronounced inhibitory role of SCN-derived VP for the circadian activity of the HPA axis (Kalsbeek & Buijs, 1992). Several routes of control seem to be incorporated in this function: direct synaptic contacts of SCN neurons on the corticotrophin releasing factor (CRF)-producing neurons of the PVN and an indirect input on these neurons via the DMH. In both instances the release of adrenocorticotrophin hormone (ACTH) from the anterior lobe of the pituitary may be controlled in a rhythmic fashion leading to rhythmic production and release of corticosterone from the adrenal. In addition, there is a multisynaptic pathway via the PVN and the intermediolateral column neurons of the spinal cord to the adrenal as demonstrated by transneuronal virus tracing from the adrenal (Buijs et al. 1999). The functional significance of this SCN-adrenal connection was demonstrated by a fast light-induced decrease in plasma corticosterone that could not be related to a decrease in ACTH, and was not observed in SCN-lesioned rats. Microdialysis studies in the rat DMH not only revealed a pronounced inhibitory role of SCN-derived AVP on the activity of the HPA axis, but also the existence of an as yet unidentified stimulatory factor (Kalsbeek et al. 1996b; Kalsbeek et al. 1996a). The degeneration of AVP neurons of the SCN during the course of aging and in Alzheimer’s disease (Swaab et al. 1985b; Liu et al. 2000) is, therefore, thought to be an important contributing factor for the elevated cortisol levels and a decreased amplitude of circadian rhythm (Swaab, 1999). In the second place, two animal experimental studies have demonstrated that adrenalectomy and dexamethasone can affect the levels of AVP or AVP mRNA in the SCN (Larsen et al. 1994; Reiter et al. 1995). The latter observations in rats suggest that the disturbance of circadian rhythms in patients treated with glucocorticoids may be due to the action of these hormones on SCN function (see chapter 5). Although the cortisol rhythm is not affected by sleep deprivation or by an abrupt shift in the sleep period, temporal relationships between sleep and cortisol have been found. Light sleep, awakening and decreasing slow wave sleep are associated with higher hormone levels, while deep sleep is related to lower cortisol levels. Sleep impairment is also one of the major side effects of glucocorticoid therapy (Moser et al. 1996; Wolkowitz, 1994; Gift et al. 1989; Braunig et al. 1989). The mechanism responsible for sleep disturbances in glucocorticoid-exposed patients is not very well understood, but hormone-induced alterations in SCN function are presumed to be a major factor. Various studies indicate that the HPA axis is activated during aging (Raadsheer et al. 1994; Dodt et al. 1991) and even more so in dementia (Swaab et al. 1994; Dodt et al.
1991; Martignoni et al. 1990). Although activation of the HPA-axis is not always reflected in increased basal plasma cortisol levels (Franceschi et al. 1991), it is apparent in approximately 50% of the patients with dementia from the non-suppression of plasma cortisol following dexamethasone administration (Davis et al. 1986).

We argued that the degenerative changes in the SCN may be the biological basis of circadian disturbances and contribute to the increased cortisol levels in aging and AD. In order to establish whether high glucocorticoids levels may in turn affect the function of the human clock, and so be responsible for the sleep deficiency of glucocorticoid-treated patients, we measured the amount of AVP mRNA in the SCN of glucocorticoid-treated patients.
V Circadian rhythm disturbance in various diseases.

Daily rhythms are important factors in the various diseases that come to expression. Ischemic stroke and intracerebral hemorrhage show a postawakening morning peak, whereas a similar rhythm for subarachnoid hemorrhage has been recorded in hypertensive (but not in normotensive) patients (Roman et al. 1997; Okin et al. 1997). It is of interest that generally a significant nocturnal blood pressure fall is observed (dippers) while in some essential hypertensives this nocturnal fall in blood pressure is absent (non-dippers) (Coca, 1994). The onset and symptoms of asthma attacks, coronary infarction, angina pectoris, and ventricular tachycardia are circadian-phase dependent. Irregular sleep-wake syndrome is a disorder in which the circadian pacemaker is probably disturbed. Sleep onset insomnia and early morning awakening insomnia may be caused by a delay and an advance of circadian rhythm, respectively. Sleep onset insomnia goes together with a delay of the peak/acrophase of the body temperature rhythm. Early morning awakening insomnia has significant phase advances of 2-4h for temperature and melatonin. The therapeutic implication of this finding would be that early morning awakening insomnia could be treated effectively by evening bright light therapy, which would cause a phase delay in the circadian rhythm (Lack et al. 1996). In addition, circadian disturbances are found in depression and aging, where the SCN is affected (Zhou, submitted 2000). Age-related changes have been found in rhythmic levels of cortisol, AVP, blood pressure, and many other endocrine circadian rhythms in human beings (Magri et al. 1997b; Asplund & Aberg, 1991; Touitou et al. 1995; Forsling et al. 1998), while the amplitude of the body temperature rhythms appears to be only slightly affected (Touitou, 1997; Monk et al. 1995). From temporal isolation experiments it appeared that there is a negative relationship between the period of the clock and the age of the individual. In addition, 80% of subjects in the 50-80 years range show a spontaneous internal desynchronization that may affect sleep patterns and other aspects of biological aging (Mirmiran et al. 1992). Changes in circadian rhythms are frequently associated with a reduction in nighttime sleep quality, a decrease in daytime alertness and an attenuation in cognitive performance (Myers & Badia, 1995). Furthermore, patients sleep in rooms where the corridor lights are kept on during the night. The fragmented sleep-wake pattern which occurs in senescence is even more pronounced in AD (Mirmiran et al. 1992; Witting et al. 1990; Bliwise et al. 1995; Ancoli-Israel et al. 1997). In AD, disruptions of the circadian rhythms are often so severe that they are even thought to contribute to mental decline and to increase agitation during the day and restlessness at night. The degenerative changes in the retina and optic nerve associated with Alzheimer's disease also decrease the light information to the circadian system. All these factors can affect the synchronization of the brain's biological clock to 24-hour environmental cues.
VI Biological rhythm disorders in aging and Alzheimer's disease (in press)

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From the moment of conception to the moment we die, biological rhythms play a prominent role in our lives. Whereas environmental periodic phenomena only entrain biological rhythm to the environmental changes, it is the organism itself that creates these rhythms. The endogenous biological rhythms enable the organism to anticipate rhythmic changes in the environment, which is, consequently, an important adaptive process. A substantial number of human beings, i.e., some 33%, exhibit a desynchronization of their internal time structure. This goes, e.g., for body temperature and blood pressure (1). Several factors outside the biological block may be involved in such disorders. Individual circadian desynchronization of various circadian rhythms has been documented during isolation experiments without time cues and a genetically controlled variability has been suggested (2).

Circadian rhythms may be disturbed by various factors, such as hypothalamic tumors in the region of the suprachiasmatic nucleus (SCN) (3, 4). In addition, when third-ventricle tumors cause ventricular obstruction with consequent increased intracranial pressure and hydrocephalus, circadian temperature fluctuations disappear. Moreover, circadian disorders occur in aging and Alzheimer's disease (AD), in depression, and following glucocorticoid administration (see below), and circadian rhythms are altered by oral contraceptives (5). Totally blind people often lack the entraining effects of light and may show free-running temperature, cortisol and melatonin rhythms. Because of their drifting intrinsic periodicity, totally blind people may also suffer from recurrent sleep disturbances (6, 7). Surprisingly, some blind people maintain circadian entrainment and show light-induced suppression of melatonin secretion, despite the apparently total lack of pupillary light reflexes, and with no conscious perception of light (8). It has been proposed that in these patients the retinohypothalamic pathway that innervates the SCN would still be intact, but the exact nature of the circadian photoreception in these patients is not known.

It might be of practical importance to recognize these patients, since enucleation of the eyes might cause recurrent insomnia and other symptoms associated with the loss of entrained circadian rhythms. The observations in patients with a tumor in the SCN region (3, 4) as well as those in blind people emphasize the importance of the light-dark cycle for synchronization and of the SCN for circadian rhythms in the human species.

Disorders of clock function and circadian rhythms
Irregular sleep-wake syndrome is a disorder in which the circadian pacemaker is prob-
ably disturbed. In addition, circadian disturbances are found in situations in which the SCN is affected: depression, aging and AD. In a patient with multiple system atrophy a decrease in the number of AVP neurons in the SCN was found, accompanied by nocturnal polyuria (9). Refusal to attend school in Japanese children and adolescents was found to go together with desynchronization of their biorhythms, particularly the circadian rhythm of body temperature and sleep-wake (10).

Circadian fluctuations of symptoms are also found in a number of diseases. Tremor, e.g., in Parkinson patients, shows strong circadian fluctuations with a clear decline during the night (11). Daily rhythms are important factors in the expression of various diseases, e.g., in ischemic stroke and intracerebral hemorrhage, that show a post-awakening morning peak, whereas a similar rhythm for subarachnoid hemorrhage has been recorded in hypertensive, but not in normotensive, patients (12). The incidence of subarachnoidal hemorrhage is related to circadian blood pressure variation in hypertensive patients, as is the case with the diurnal rhythms observed with strokes and myocardial infarctions. Normotensive individuals, in contrast, have a random 24-h distribution of subarachnoidal hemorrhage (13). In this respect it is of interest that a significant nocturnal blood pressure fall is generally observed (dippers); in some essential hypertensives this nocturnal fall in blood pressure is absent (non-dippers) (14). The normal circadian pattern in vasopressin blood levels with higher levels during the night is absent in nocturnal diuresis (15), in hepatorenal syndrome, also known as functional renal failure of liver cirrhosis (16), and in a case of Shy-Drager syndrome (multi-system atrophy) that exhibited nocturnal diuresis (9). Tardive dyskinesia and progressive dystonia with diurnal variation (Segawa's dystonia) after awakening worsen with time. Acute dystonic reactions to neuroleptics are more likely to occur in the afternoon and evening. Migraine headaches seem to be more frequent in the morning. Headaches and seizures may be associated with sleep, cluster headache may be linked with rapid eye movement (REM) sleep (12) and migraine may start during nocturnal sleep (17). Some authors even state that the SCN may be the site where the migraine attack is initiated (18). In addition, a hypnic headache syndrome of the elderly has been described, characterized by recurrent nocturnal headaches that awaken patients from sleep at a consistent time each night and respond to treatment with lithium carbonate (19). A 50-year-old patient has been described with stupor occurring in a clear circadian pattern, i.e., almost every afternoon, for a period of 10 years. Such a stuporous attack lasted from 5-8 p.m. (20).

AGE AND ALZHEIMER'S DISEASE
Age-related changes have been found in rhythmic levels of cortisol, vasopressin, blood pressure, and many other endocrine circadian rhythms in human beings (21,22,23,24,25). But the amplitude of the body temperature rhythms appears to be only slightly affected (26,27). From temporal isolation experiments it appeared that there is a negative
relationship between the period of the clock and the age of the individual. In addition, 80\% of subjects in the 50-80 years range show a spontaneous internal desynchronization that may affect sleep patterns and other aspects of biological aging (28). Changes in circadian rhythms are frequently associated with a reduction in nighttime sleep quality, a decrease in daytime alertness and an attenuation in cognitive performance. An increased level of physical activity improves circadian rhythmicity in healthy elderly people as was found following a 3-month fitness training period (29). The fragmented sleep-wake pattern which occurs in senescence is even more pronounced in Alzheimer’s disease (AD) (28, 30, 31, 32, 33). In AD, disruptions of the circadian rhythms are often so severe that they are even thought to contribute to mental decline. The melatonin levels and nadir values of plasma cortisol are related to mental impairment (24). Demented patients frequently suffer from sundowning, characterized by an exacerbation of symptoms indicating increased arousal in the late afternoon, evening or night. Sundowning is considered to be a chronobiological disturbance (34). Disruption of the sleep of the caregiver due to nocturnal problems of the patient is a more important reason for having a demented patient admitted to a nursing home than cognitive impairment (35). Changes in the SCN and pineal gland are considered to be responsible not only for the disturbed circadian rhythms in hormones and sleep-wake behavior, but also for behavioral disorders in elderly people and AD patients.

SUPRACHIASMATIC NUCLEUS (SCN)

The SCN is the major circadian pacemaker of the mammalian brain and coordinates hormonal and behavioral circadian rhythms (36). The vasopressin subnucleus of the SCN has a volume of 0.25 mm$^3$ on each side (37). The estimation of the SCN depends strongly on the sensitivity of the technique used. Following microwave treatment of sections the staining of vasopressin and VIP becomes more sensitive. The volume of the vasopressin SCN subnucleus increased 2.4 times and that of VIP 4 times, the number of vasopressin neurons increased by 7.6\% and the number of VIP neurons 8 times. The neurons that were visible without microwave treatment were localized mainly in the central part of the SCN, whereas the neurons that became visible only after microwave treatment could be found in the peripheral area of the subnucleus. This suggests that the vasopressin and VIP neurons in the central part of the SCN contain more peptides, possibly because they are more active than the peripheral ones (38).

Animal experiments have shown that lesions restricted to the SCN make them totally arrhythmic, while transplantation of a fetal SCN may restore circadian rhythms in such lesioned animals (39, 40, 41). A few clinical observations support the importance of the SCN for circadian rhythms in human. A lesion in the suprachiasmatic region of the human hypothalamus, e.g. as the result of a tumor, indeed results in disturbed circadian rhythms in human beings (3, 4, 42).
In a patient with an hypothalamic astrocytoma destroying the SCN bilaterally, reversal of the day/night rhythm of the wake/sleep pattern was also reported (43). It should be noted, though, that in that patient not only the area of the SCN, but also a large part of the hypothalamus was affected. Moore (44) briefly reported on a patient with an optic nerve glioma who had evidence of loss of rhythmicity in several functions and compression of the chiasmatic area as visualized by a CT scan. The scarce medical information given on the patients, described by Krieger and Krieger (45), with circadian disturbances as a result of a disease of the temporal lobe, pretectum or hypothalamus, does not allow conclusions on the possible direct involvement of the SCN in this type of disorder.

A relatively large number of neuropeptides has been identified in the human SCN. Neurons that are immunoreactive for vasopressin, vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY), neurotensin (NT) and somatostatin (SOM), are present in the SCN in a characteristic anatomical orientation (Fig 2; 37, 44, 46, 47). Using confocal laser scanning microscopy, Romijn et al. (48) found that a small percentage of the neurons in the human SCN colocalized vasopressin and VIP. Most, if not all, neurons in the human SCN contain the two isoforms of glutamic acid decarboxylase (GAD), GAD65 and GAD67. GABA (γ-aminobutyric acid) is colocalized with one or more peptides in SCN neurons (49). GABA is generally known as an inhibitory neurotransmitter in the brain. Wagner et al., (50) have shown, however, that SCN neurons can be excited by GABA through a GABA_a-dependent mechanism. The excitatory response to GABA is seen only during the day, when GABA opens the chloride ion channels. The membrane potential then becomes more positive and action potentials are generated. The opposite happens during the night. Then GABA acts as an inhibitory neurotransmitter. Whether GABA has similar time-dependent effects on brain areas innervated by the SCN, and what the role of the colocalizing peptides might be is yet to be investigated.

The region of the SCN that receives retinohypothalamic tract input and is therefore considered to be of importance for entrainment, is characterized by VIP neurons (44, 47). Vasopressin is found in the remainder of the SCN. It might amplify the rhythm in this nucleus by its excitatory effect during the light phase as shown by animal experiments (51) and was shown to play a role in synchronizing circadian rhythms to the light entrainable oscillator. The observation that intranasal vasopressin markedly enhanced nocturnal slow-wave sleep in human (52) should perhaps be considered in the light of the function of this neuropeptide in the SCN.

Vasopressin and vasoactive intestinal polypeptide (VIP) changes in aging, Alzheimer's disease and depression

AGING

Our earlier observations have revealed marked day-night fluctuations in the volume of the vasopressin (AVP) subpopulation and the AVP-expressing cell number of the hu-
Fig. 2 Diagram showing the organization of the human SCN. The distribution of vasopressin (VP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) neurotension (NT) neurons (large black dots) and fibers (small gray points) is shown at three levels, from rostral to caudal. (From 44, with permission).
Fig. 3 Circadian rhythm in the number of vasopressin-containing neurons in the human suprachiasmatic nucleus (SCN) of (A) young subjects (<50 years of age) and (B) elderly subjects (≥50 years of age). The black bars indicate the night period (22:00-06:00 h). The general trend in the data is enhanced by using a smoothed double-plotted curve and is represented by mean ± S.E.M. values. Note the circadian rhythm in the SCN of young people with low values during the night period and peak values during the early morning (from 55, with permission).

man SCN in relation to the seasons. In addition, seasonal fluctuations in the number of AVP expressing neurons in the SCN are present (53, 54) (Fig.4). These biological rhythms are only found in young subjects (55) (Fig. 3), which suggests a diminution of circadian fluctuations of the SCN in humans during aging. Age-related changes in circadian rhythms have indeed been reported in man as well as in other species (30, 56, 57). Fur-
Fig. 4 Annual rhythm in the number of vasopressin-containing neurons in the human suprachiasmatic nucleus (SCN) of (A) young subjects (<50 years of age) and (B) elderly subjects (≥50 years of age). The general trend in the data is enhanced by using a smoothed, double-plotted curve and is represented by mean ± S.E.M. Note the circannual rhythm in the SCN of young people with low values during the summer and peak values in the autumn period (from 117, with permission).

Furthermore, a decrease in the number of AVP cells and in the total number of cells was found in subjects aged 80-100, while these changes were even more pronounced in Alzheimer's disease patients than in controls (Fig.5; 37, 58). A quite different pattern of changes was found recently in the number of VIP-expressing neurons of the human SCN during aging (Fig.6; 59, 60, 61). In males, the number of VIP neurons in the SCN reached its
**SUPRACHIASMATIC NUCLEUS**

![Graph](image)

Fig. 5 In both presenile (n=7) and senile (n=8) Alzheimer patients the volume of the vasopressin subnucleus of the SCN (A) and the number of vasopressin-expressing neurons (B) is significantly decreased when compared to young (n=14) or old (n=9) age-matched controls. In presenile Alzheimer patients only 10% of the number of neurons expressing vasopressin in controls is found (unpublished data, D.F. Swaab). **=P<0.001, *=P<0.02** (Mann-Whitney U test).

peak value in young men (10-40 years of age). Subsequently, a dramatic decrease in the number of VIP neurons in the SCN was found in middle-aged male subjects (41-65 years of age). A significant reduction in the number of VIP expressing neurons was found in the old-age group (65-92 years of age). An age-dependent sex difference was observed in the SCN: in males of 10-40 years of age it contained twice as many VIP neurons in the SCN as in females. Due to the age-related fluctuations in VIP cell number in males, this sex difference was reversed in the middle-aged group, the females having twice as many VIP neurons in the SCN. After 65 years a significant sex difference was no longer found. Since the SCN is the clock of the brain, these sex differences may be related to sex differences that have been found in circadian control mechanisms of hamsters (62) and humans (63). In addition, it has been found in rat that VIP-expressing neurons from the SCN directly innervate LHRH neurons that are involved in reproductive functions (64). Although such a connection should be confirmed in the human hypothalamus, the sex difference in the number of VIP neurons in the SCN and the difference in the AVP subnucleus of the SCN according to sexual orientation (65,66) also suggests a possible role of the biological clock in reproduction or sexual behavior. It is also tempting to relate the
Fig. 6 Lifespan changes in the number of vasoactive intestinal polypeptide (VIP) immunoreactive neurons of the human SCN in control subjects. The SCN of young males (10 to 40 years) contains twice as many neurons as that of young females (**P<0.02). This sex difference reverses in middle-aged subjects (*P<0.04). Note the decrease in the number of VIP cells started already in middle-aged males and the significant reduction in the elderly males compared with young males (# p<0.02) (from 60, with permission).

age-related changes in the peptidergic neurons of the SCN to functional changes, e.g., in circadian rhythmicity. Although the number of AVP neurons in the SCN in 60 to 80-year-old subjects did not differ from the number found in young subjects (67), the circadian fluctuations in the number of AVP neurons in the human SCN diminished in subjects older than 50 years (55; Fig. 3). The number of AVP neurons did not decrease until after 80 years of age (37, 67), which suggests that the circadian fluctuations in AVP neurons disappear earlier in the process of aging than the number of neurons expressing AVP.

ALZHEIMER'S DISEASE

It has been proposed that the decreased AVP cell number might be the basis for circadian disturbances, not only in aging, but also, and even more so, in Alzheimer’s disease (37, 58). To establish whether the degenerative changes in the SCN of AD patients are indeed accompanied by decreased AVP gene expression of the SCN, we investigated AVP gene expression in the SCN (68). The in situ hybridization procedure on formalin-fixed paraffin-embedded material was improved to such a degree that we could, for the first
time, visualize AVP mRNA-expressing neurons in the human SCN and carry out quantitative measurements. A clear decrease of AVP mRNA was indeed found in the SCN of the AD patients. The total amount of AVP mRNA in the SCN was three times lower in AD patients than in age and time-of-death matched controls. Moreover, we found that the total number of profiles that expressed AVP mRNA in the SCN in AD patients was only 40% of that of controls. The decreased AVP mRNA levels in the SCN in AD patients corresponds well with previous reports showing a major reduction in the number of AVP immunoreactive neurons in SCN (37, 58). Since the SCN is the clock of the brain, the time of death should be considered as a possible confounding factor. We excluded this possibility by matching AD patients with control subjects who had died around the same time. Moreover, the reduction in AVP mRNA level found in AD patients was present during the entire day, whereas no clear diurnal rhythm of AVP mRNA-expressing neurons in the SCN was observed in AD patients. It is interesting to note that the day–night fluctuations in the amount of AVP mRNA and in the total number of profiles in the SCN were only observed in controls under 80 years. No diurnal fluctuations in AVP mRNA were observed in controls over 80 years. This finding also agrees with the disappearance of circadian rhythmicity in the number of AVP immunoreactive neurons in the SCN of elderly people (55). The low amount of AVP mRNA is also reflected by a decreased number of AVP neurons in the SCN in subjects older than 80 years (37). Recently, a significant decrease of cerebrospinal fluid (CSF) melatonin was found in the control subjects who were older than 80 years (69). We propose that degenerative changes in the SCN and a decrease in melatonin synthesis may underlie the common sleep disturbances among elderly people. In contrast to what was seen in the controls, no significant diurnal variations in any of the SCN parameters were observed in the AD group. Several studies have indeed shown the presence of circadian rhythm disturbances in aging and AD (57, 70). Our observations support the idea that these behavioral disturbances most probably have their basis in a decreased activity of the SCN in this disorder. AVP is one of the major neuropeptides in the SCN and is involved in the synchronization of the circadian rhythm to the light/dark cycle (71). In addition, AVP may amplify the rhythm in this nucleus (51). On the basis of the observations in human and rat one may expect that stimulation of the circadian system may have important therapeutic consequences for AD patients and elderly people. Indeed, exposure to bright light was found to have a positive effect on both the phase and amplitude of the circadian pacemaker (8). Appropriately timed exposure to bright light may thus be used in the treatment of circadian rhythm-related behavioral disturbances such as sleep disorders in AD patients and elderly people (70, 72).

DEPRESSION IN AD
Depression is a common symptom in AD patients. Depression and dementia have a
number of symptoms in common. In order to control for symptom overlap between dementia and depression we used the Cornell scale, which was specifically developed for the assessment of depression in all stages of dementia. In both AD and depression a relationship between the pathology of the SCN and dysfunction of biological rhythms may be present. Recently, we found a significantly lower amount of AVP mRNA in the SCN in depression (J.N.Zhou, et al. submitted). Based on these findings we considered the possibility that depressed AD patients might have the lowest amount of AVP mRNA, which turned out not to be the case. The amount of AVP mRNA or the total number of profiles with AVP mRNA in the SCN of AD patients with depression was not different from AD patients without depression (68). The reason could be that the AVP mRNA values in AD patients without depression were already extremely low. In conclusion, we found that, independent of the presence or absence of depression, AD patients showed a strongly decreased production of AVP in the SCN, which may be the biological basis for diurnal behavioral disorders and for the beneficial effect of light therapy.

*Decreased melatonin levels in relation to aging and Alzheimer's disease*

Only a small number of hypothalamic nuclei are directly innervated by the SCN (73, 74). But indirectly, by means of melatonin from the pineal gland, this nucleus imposed circadian fluctuations on many more brain structures. The pineal gland contains the melatonin secreting pinealocytes. The pinealocytes are arranged in cords or lobules embedded in a matrix of neuroglia surrounded by septa. The pinealocytes have club-like endings. In the pineal, concrements, also known as acervulli, corpora arenacea, brainsand, or psammoma bodies, are present. They contain hydroxyapaties and calcium phosphate. The incidence of concretions in the pineal increases with age (75). Serum melatonin levels are high at night and low during the day (76). The main environmental stimulus for the rhythmic production of melatonin is light intensity. During darkness, norepinephrine is released from the sympathetic nerve endings in the pineal gland to activate $N$-acetyltransferase, the enzyme which catalyses the rate-limiting step of the synthesis of serotonin and melatonin from tryptophan. The adrenergic-cAMP regulation of $N$-acetyltransferase activity is mediated by rapid reversible control of selective proteasomal proteolysis (77).

The pineal gland is innervated by the SCN by a multisynaptic pathway via the paraventricular nucleus, the spinal cord and the superior cervical ganglion, and melatonin is produced and released causing circadian fluctuations in many brain structures and functions (53). In turn, melatonin elicits two distinct, separable, effects on the SCN, i.e., acute neuronal inhibition and phase shifting (78). Specific high-affinity melatonin binding sites have been observed consistently in the human SCN. In contrast, such binding was detectable in the pars tuberalis of the pituitary in only one out five human subjects. Melatonin binding was also detected in the pars distalis of several subjects, but with an
inconsistent distribution (76). Melatonin receptors are present in the SCN area from the 18th week of gestation onwards (79,80). A family of three subtypes of melatonin receptors has been found (81), but their exact distribution and function in the human brain awaits further research.

It should be noted that melatonin is not only produced in the pineal, but also in, e.g., the retina, Harderian gland and in the gut mucosa (82). In addition, if melatonin is used against, e.g., jet lag or circadian rhythm disturbances in blind people (83) or in Alzheimer patients one should be aware of possible adverse drug reactions (84).

Alterations in melatonin levels in development, aging and Alzheimer's disease

DEVELOPMENT
Plasma melatonin levels show maximum amplitudes around age 7. These levels decline with age (85,86). The pineal hormone 5-methoxytryptophol plasma levels show age and sex differences. Plasma levels increase in boys and decrease in girls from the age of 8 onwards (87), a pattern that is the reverse of the one we found for the number of VIP-expressing neurons in the SCN (60).

The pattern of changes in 5-methoxytryptophol in girls may have a permissive effect on puberty (87). The idea that the pineal gland may affect puberty dates back to 1898 when Heubner described a 4.5-year-old boy with precocious puberty and a non-parenchymal tumor that had destroyed the pineal gland (88). However, effects of pineal region tumors on puberty may also be due to local pressure of such tumors on the hypothalamus. On the other hand, a 21-year-old male patient described by Puig-Domingo et al. (89) supports the idea that melatonin might play a crucial part in reproduction in human beings. When the patient’s melatonin levels were 15-20 times higher than normal, the patient’s pituitary-gonadal function, including sperm production, was disturbed. Full sexual capability was restored when the melatonin secretion gradually decreased. The patient’s hypogonadotrophic hypogonadism, in fact an extreme form of delayed puberty, was thus probably caused by hypermelatoninemia. Melatonin indeed induces decreased serum LHRH levels and increased prolactin levels at pharmacological doses (88). Following testosterone replacement therapy in hypogonadal men, the high melatonin levels decreased both during the day and at night. The case described by Puig-Domingo et al. (89) also supports the view that pineal calcification is a result of hyperactivity of the pineal and not a sign of inactivity or atrophy.

AGING
The production of melatonin declines with increasing age and age-related diseases. In some patients this goes together with clinical symptoms of rhythm disturbances, such as sleep-wake disturbance (27). Whether AD patients may indeed benefit from chronic supplementation of melatonin should be investigated. It is also proposed that the re-
response of the circadian system to environmental (Zeitgeber) signals diminishes with aging, and that when the melatonin rhythm deteriorates during aging, other circadian rhythms likewise weaken and become desynchronized (90). In addition, the hypothermic response to melatonin is markedly blunted and inconsistent in aged individuals. In postmenopausal women the effect of melatonin on cerebral blood flow is reduced or absent (91). Concerning the changes of plasma melatonin observed in elderly people, the mechanism responsible for the reduction of melatonin secretion in aging is not very well understood. Alterations in the SCN (37, 60) may be a major factor. Interestingly, a significant decrease of CSF melatonin was found in the control subjects who were older than 80 years. The decreased number of AVP neurons in the SCN was also found in the subjects older than 80 years (37, 67), suggesting that the changes in the SCN and pineal are related. Structural changes of the pineal, such as the calcifications or the variations of melatonin clearance, do not seem to play an important role in the decrease of plasma melatonin levels in elderly subjects (92, 93). Nocturnal melatonin secretion is modulated by noradrenalin through β-receptors (94). Therefore it may be of importance that an impairment of catecholaminergic pathways occurs with aging in the CNS (95). The effect of a decline in the CSF production rate or turnover with aging (96, 97, 98) on CSF-melatonin levels in aging and Alzheimer's disease is not known. In our study (69, see below) a daily rhythm of melatonin levels in postmortem ventricular CSF was not observed, either in controls or in AD patients. This may well be due to the fact that our CSF samples were obtained postmortem from hospitalized patients. It has been reported that hospitalized patients have significantly higher daytime plasma melatonin levels, an earlier nocturnal rise, and a more variable timing of their secretion profiles (99, 100). Artificial and supplementary natural lighting in the hospital may not be sufficient to suppress melatonin secretion adequately during daylight hours nor act efficiently to entrain day/night secretion of melatonin in a physiologically circadian manner. This problem may exist particularly in humans. Room light of low intensity, which is sufficient to suppress melatonin secretion in other mammals, failed to do so in humans (101). Another reason for the lack of an overall circadian rhythmicity in CSF levels of melatonin may be that in spite of the reproducible pattern observed from day to day in the same individual, a very large interindividual variation was observed (102, 103). In our study only one data point per patient was available for obvious reasons. In addition, a great variety of pathological conditions and disease states have been associated with alterations in pineal function and 24-hour melatonin profiles (104, 105, 106). The normal range for daytime and night-time plasma and CSF levels is thus very large, especially in the hospitalized patients from which we obtained postmortem CSF. The day-night difference for melatonin levels can vary widely for various reasons. The observed decrease of ventricular CSF-melatonin levels with aging in controls supports other reports on plasma melatonin changes (27, 88). Age of the subject had a significant effect on the day/night varia-
tion of pineal melatonin level, the rhythmicity being lost in the older age group (104). The decline in the production of melatonin with age agrees with previous reports (104, 105, 107, 108), while in the older age group also SCN changes were observed (37).

ALZHEIMER’S DISEASE

Interestingly, the degree to which the nocturnal melatonin levels decreased in AD was reported in some studies to be related to the severity of mental impairment in demented patients (24, 110). The data in the literature concerning plasma melatonin levels in dementia are, however, discordant. Earlier studies did not report differences in plasma or pineal melatonin levels between demented and elderly subjects (104, 110, 111), nor did Magri et al. (24) find a difference in plasma melatonin levels of 6 demented patients compared with normal elderly subjects. However, a decrease in nocturnal plasma melatonin levels was observed in senile AD patients (24, 112). In addition, decreased pineal melatonin levels were found in aging and AD (104). The discrepancies between the studies on melatonin levels may be attributed to differences in the age of subjects, to the use of in- or outpatients, or to severity and type of dementia, which also varied across studies. The subjects used in the present study were neuropathologically confirmed AD and control subjects. In our recent studies we found markedly lower melatonin levels in ventricular CSF of AD patients. Melatonin levels were 5-fold lower in AD patients than in age-matched controls. Interestingly, the decreased CSF melatonin levels we observed, coincide with the general disturbance of circadian rhythms in AD, e.g., in sleep-wake, body temperature and rest-activity and with the degeneration of the SCN in aging and AD (37, 55, 58). Furthermore, demented patients tend to be exposed to less environmental light than healthy people (72). It has been reported that bright light therapy, an interference presumed to stimulate the SCN directly, was effective for sleep and behavior disorders in elderly patients with dementia (70, 112). These observations support the idea that degeneration of the SCN in AD is the central phenomenon in these changes.

Recent studies have indicated a significant association between the Apo-E type and AD. Apo-E is a 34-KDa protein that plays a key role in the regulation of the metabolism of lipids and has three major isoforms (ε2, ε3 and ε4). The Apo ε-4 genotype is a risk factor for AD (113, 114, 115) and it is likely that this will to some degree be reflected in the neuropathology and neurochemistry of this disease. Indeed, ApoE immunoreactivity has been found in senile plaques, cerebral vessels and neurofibrillary tangles in AD. An interesting finding of our study is that CSF-melatonin levels from ApoE-3/4 genotype patients were significantly higher than those from ApoE-4/4 genotype, suggesting again a relationship between melatonin levels and signs and symptoms of AD.

Our finding of the decreased CSF melatonin levels suggested that melatonin may indeed be involved in the symptoms of AD. The impairment of nocturnal melatonin secretion is related to mental impairment (24). Moreover, it was reported that melatonin
inhibits the progressive formation of β-sheets and amyloid fibrils in vitro (116). Whether supplementation of melatonin may indeed improve behavioral disturbances in AD patients should be investigated.

References Chapter1, VI
GENERAL INTRODUCTION


VII Scope of this thesis

The presence of pronounced circadian rhythms of sleep-wake, temperature and hormones is regarded as a basic necessity for optimum physical and mental functioning. During aging and particularly in Alzheimer's disease, a variety of circadian rhythms show signs of organizational decay. Changes in the SCN and pineal gland are considered to be responsible not only for the disturbed circadian rhythms in hormones and sleep-wake behavior, but also for related behavioral disorders in elderly people and AD patients. We hypothesized that decreased synthesis of AVP in the SCN, the reduction of melatonin levels from the pineal gland and increased corticosteroid levels that act on the SCN may be factors involved in the disorder of the biological timing mechanisms and in the pathogenesis of AD changes. In the present thesis we therefore combined the staging of AD according to Braak and Braak for the distribution of AD changes and differentiated semi-quantitative score of neurofibrillary tangles (NFT), neuritic plaques (NP) and disruption of the neuropil (DN) to estimate the severity of AD changes, in order to investigate whether there is a relationship between CSF melatonin levels and the neuropathological hallmarks of AD (chapter 2,3) and whether there is a reduced AVP production in the SCN as measured by in situ hybridization (chapter 5). In addition, we investigated the possibility that a decrease of cerebrospinal fluid melatonin levels may be an early event in the development of AD, occurring even before the clinical symptoms start to become apparent (chapter 3). In aging subjects and even more so in AD patients, the decreased activity of the SCN is accompanied by higher cerebrospinal fluid cortisol levels. Since, moreover, circadian rhythm impairments are major side effects of glucocorticoid therapy and are also frequently seen in elderly subjects and AD patients, we investigated whether the AVP mRNA expression in the human SCN was indeed affected by increased glucocorticoid levels (chapter 6).

In order to see whether increased glucocorticoid levels may not only affect circadian rhythm in old and demented people but also in young subjects, we investigated whether academic stress may also affect the circadian rhythms in healthy students (chapter 7). Since the decreased melatonin levels were found to be related to early AD neuropathological changes (chapter 3), we want to study in the future whether low melatonin levels in plasma, or, even better, in saliva, may indeed be a marker for very early AD changes. As the first step, we measured circadian rhythm of saliva melatonin in 4 age groups of healthy subjects (chapter 4). The conclusion of the present thesis is that both decreased melatonin and increased cortisol levels may also contribute to the circadian disorders in aging and AD. There are indications that decreased melatonin levels may contribute to the pathogenesis of AD and be an early maker of the AD process. This possibility will be investigated in the near future by following saliva melatonin levels in patients with early memory problems up to the moment they clearly develop possible or probable AD.