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

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Citation for published version (APA):
Circadian rhythms are present in many physiological and behavioral phenomena in most, if not all, species. Changes in these rhythms often develop in aging, and even more so in Alzheimer's disease (Touitou et al. 1981; Swaab et al. 1985a; Swaab, 1998; Swaab, 1999). Whether these rhythm alterations are only a consequence of, or even a causal factor in the aging process is still debated (Touitou & Haus, 2000). Aging affects different brain structures and systems in a differential way. The process of aging is not only accompanied by degeneration; activation of neuronal systems is also found (Swaab, 1995; Coleman & Flood, 1987; Swaab, 1991). A relevant example for the present study is the age-related increase of CRH neurons (Raadsheer et al. 1995) leading to increased cortisol in aged subjects (Swaab et al. 1994). But the age-related functional changes in circadian organization in humans are generally thought to be associated with degenerative alterations in the suprachiasmatic nucleus (SCN) and decreasing melatonin levels, which leads to a disruption of the circadian system (Touitou et al. 1981; Swaab et al. 1985a; Liu et al. 2000; Swaab, 1998; Swaab, 1999). On the other hand, it was shown that disruption of the circadian timing system may happen relatively early in life, well before any dramatic neuronal changes become manifest in the circadian system.

The present study focussed on three possible factors that may play a role in circadian rhythm disturbances in aging and Alzheimer’s disease, (i) decreased melatonin secretion, (ii) decreased vasopressin production in the SCN and (iii) increased glucocorticoid secretion. To investigate the importance of this last factor we studied the effect of increased exogenous and endogenous corticosteroids on circadian rhythmicity.
1 Methodological considerations

CSF changes in relation to aging and Alzheimer's disease

Currently the definitive diagnosis of AD still requires histopathological examination of the brain. A biomarker for AD based upon, for instance, CSF measurements could be useful, not only for confirmation of the diagnosis of probable AD, but also for monitoring the progression of the disease and the efficacy of potential therapies. In the present study we found decreased levels of melatonin in postmortem CSF (chapter 2) that appeared to occur extremely early in the Alzheimer process (chapter 3). In addition, our group had already found earlier increased postmortem CSF levels of cortisol in presenile Alzheimer patients (Swaab et al. 1994). The decreased melatonin levels we observed in the very first stages of AD (chapter 3) was surprising. It is, however, of importance to consider the factors that may possibly have confounded our results. CSF measurements are complicated by the fact that each component of the CSF circulatory system is subject to changes in senescence and AD. The rate of CSF production in healthy people of advanced age has been found to be about half of that of their younger counterparts (May et al. 1990), while the CSF in young healthy people is turned over three to four times per day. In aged people the turnover rate is about half of that observed in young people (Galasko et al. 1997; Rubenstein, 1998). Brain volume starts to decrease after the age of 40, while the CSF volume increases from that age onwards (Matsumae et al. 1996). The total ventricular CSF is increased by 40% during aging (Murphy et al. 1992) and by 99% in AD (Tanna et al. 1991; Wahlund et al. 1993).

Senescence also leads to changes in the composition of CSF. The age-related increase in CSF alpha₂-macroglobulin levels is an example. Although plasma levels of alpha₂-macroglobulin do not change throughout a person's lifetime, the mean CSF levels increase steadily with age. This increase has been attributed to an age-related loss of integrity of the blood-brain barrier (Moore, 1997). Other CSF components that increase in the elderly and in age-related dementia include tau protein, ceruloplasmin and ferritin (Galasko et al. 1997; Loeffler et al. 1994; Kuiper et al. 1994; Trojanowski, 1996). Also, CSF cortisol increases with age due to an increased CRH activity (Swaab et al. 1994), while a loss of blood-brain barrier integrity is not presumed to be the base for this change. Other compounds, such as β-amyloid 1-42 (Aβ42), decrease in CSF (Galasko et al. 1998). Some cross-sectional studies have suggested that decreased Aβ and increased tau levels in CSF correlate with the severity of dementia in AD patients (Nitsch et al. 1995; Tato et al. 1995). Various studies indicate that the circadian rhythm of melatonin is disturbed during aging (Touitou et al. 1981; Sack et al. 1986; Ferrari et al. 1995; Iguichi et al. 1982). There are a few data available on plasma melatonin levels in dementia (Uchida et al. 1996; Magri et al. 1997a) and information on melatonin levels in CSF was totally lacking. Because of the presumed protective effect of melatonin on the aging brain we determined CSF mela-
tonin levels and found lower melatonin levels in aging and strongly diminished melatonin levels in AD in an APO-E genotype-related way (chapter 2,3). This result is most probably not confounded by the senescent changes in volume and turnover of the CSF. A possible confounder for the observed decrease in CSF melatonin levels would be a strong increase in CSF volume or turnover between Braak stages 0 and 1, or between the total neuropathology scores 0 and 1-6. Unfortunately, no information is available on the volume changes in CSF in the very early AD stages in aged controls where we found the strong decrease in melatonin levels (chapter 3). This should be a topic for further study. However, pleading against the possibility of dilution of CSF as a confounder are the observations that the CSF fluid production rate is decreased and the total protein concentrations in CSF are higher in the elderly than in young individuals (May et al. 1990; Rubenstein, 1998). In a three-year follow-up study of CSF tau and β-amyloid-42 concentrations in AD, no consistent changes in CSF tau levels were found (Tapiola, et al. 2000). A strong argument in favor of the idea that melatonin production is decreased resulting in a decrease of ventricular CSF-melatonin levels in aging and dementia is that others have reported a decrease in plasma melatonin under these conditions (Magri et al. 1997a; Ferrari et al. 1997). The possibility that melatonin levels indeed decrease very early in the Alzheimer process should be further investigated in saliva.

A number of additional factors might theoretically also interfere with the results of our study. We did not find a significant correlation between ventricular CSF-melatonin levels in controls or AD patients on the one hand, and postmortem delay or pH of CSF on the other. There is, consequently, no reason to presume that these factors may have influenced our results (chapter 2,3).

A general problem for the relevance of CSF markers for monitoring disease progression or performance of putative therapies in CSF is that it is, for obvious reasons, not possible to perform regular lumbar punctures. Salivary melatonin is thought to reflect the changes in plasma melatonin levels closely (Nowak et al. 1987) and may, therefore, be an excellent method of monitoring changes in melatonin secretion in early stages of AD. There is a linear relationship between CSF cortisol and plasma cortisol (Swaab et al. 1994, Erkut et al. 2000 submitted) and between serum cortisol and saliva cortisol (Umeda et al. 1981). But the relationship between CSF and plasma, CSF and salivary melatonin is lacking and is a subject for future study.

Scoring the severity of the neuropathological AD changes

One of the aims of the present thesis was to investigate the possible role of melatonin in the pathogenesis of AD. Therefore, we first needed to establish whether there is a relationship between melatonin levels and neuropathological AD changes. Gold et al (2000) compared the cognitive impairment assessed by the Clinical Dementia Rating (CDR) scale and the Braak neuropathological stages of neuritic Alzheimer lesions, and observed
a strong positive correlation between CDR scores and Braak staging (Gold et al. 2000). About Braak score for amyloid: there is a big gap between Braak A and B, and it is also quite difficult to distinguish between Braak B and C. In the present thesis we found that the Braak stages for tangles (Braak & Braak, 1996) were negatively correlated with CSF melatonin levels (chapter 3). As Braak's stages indicate the extent of the neuropathological changes over the brain (Braak & Braak, 1996) and not the severity of the changes in the various cortical areas affected, a modified Braak staging for cortex (MBSC) (as developed by Dr. Wouter Kamphorst, Department of Pathology, Free University, Amsterdam) was performed in formalin-fixed Bodian stained sections of the frontal, temporal, parietal and occipital cortex, indicating the amount of neurofibrillary tangles (NFT), neuritic plaques (NP) and disruption of the neuropil (DN) in those 4 cortical areas. This score was also used earlier to match for the severity of neuropathological AD changes in AD with and without depression (Hoogendijk et al. 1999a; Hoogendijk et al. 1999b).

The criteria of MBSC were as follows: In each cortical area of AD patients and controls changes were separately scored as 0=absent, 1=present but less than moderate, 2=moderate (i.e. two or three neurofibrillary tangles, two or three neuritic plaques or 30-60% of the normal network replaced by neuropil threads per 0.4 mm²) and 3=more than moderate. Here, we illustrate the results of this scoring system for one moderately affected AD patient (#99140) (Table 1); Macroscopic examination of the patient showed clear frontotemporal atrophy, that reached maximum values mediotemporally, and a severely dilated ventricular system. The substantia nigra was normally pigmented, but the locus ceruleus was hardly visible. In the temporal, frontal and parietal cortex many NPs, NFTs and DN were found. In the occipital part, there was a substantial number of NPs, NFTs and moderate numbers of DN.

By using MBSC we found that the earliest AD changes that occur in the temporal cortex, where the AD process starts, were correlated with diminished melatonin levels. Those controls that did not have any NFT or NP had much higher CSF melatonin levels than

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<th>Area</th>
<th>NP</th>
<th>NFT</th>
<th>DN</th>
<th>Total score in each area</th>
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<tr>
<td>Frontal cortex</td>
<td>3</td>
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<td>3</td>
<td>9</td>
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<tr>
<td>Parietal cortex</td>
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<td>Occipital cortex</td>
<td>2</td>
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<td>Total score in cortex</td>
<td>11</td>
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<td>33</td>
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NP: neuritic plaques; NFT: neurofibrillary tangles; DN: disruption of the neuropil

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the controls that had a few NFTs or NPs in the temporal cortex. In the other cortical areas, i.e. frontal, parietal and occipital, we did not find such a relationship between decreased CSF melatonin levels and the occurrence of neuropathological changes in aged controls (chapter 3). There was a negative correlation in CSF melatonin levels not only with the Braak stages, but also with MBSC score.

Parameters influencing in situ hybridization

The in situ hybridization procedure on formalin-fixed paraffin-embedded material was improved to such a degree that we could show and quantify, for the first time, AVP mRNA expressing cell profiles in the human SCN (chapter 4). The amount of AVP mRNA from three patients (96057; 95092;95054) could be determined in a very reproducible way in two experiments that were performed with a 2-year interval, in 1998 and 2000 (Table 2).

The expression of AVP mRNA in the SCN may be influenced by a number of parameters, i.e. PMD, fixation time (FD), pH and the clock time of death. Earlier experiments showed no differences in the amount of AVP mRNA in SON and PVN between a short fixation time of 10 days and a longer fixation time of 35 days (Lucassen et al. 1995). However, in a pilot study we found that a much longer fixation time (more than 298 days) sharply reduced the signal of AVP mRNA expression in the SCN. This finding was recently confirmed in a series of experiments and also revealed that the AVP mRNA can be made available after very long fixation times by microwave pretreatment (U Unmehopa, unpublished observation). For the experiments of the present thesis, the subjects with long fixation times (>130 days) were excluded.

As far as PMD is concerned, the half life of AVP mRNA in post mortem rat brain was shown to be approximately 16 h (Noguchi et al. 1991). On the other hand, it remained possible to localize several mRNAs in the human brain after PMDs of up to 40h (Mengod et al. 1990). Lucassen et al (1995) reported that the AVP mRNA signal in cryostat tissue decreases during the first hours of PMD with a rather strong decrease in the first 3 hours, and no further decrease in signal after 6 hours of PMD. In paraffin tissue the rapid decline in the first hours postmortem as seen in cryostat sections does not occur, so that no

<table>
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<th>NBB</th>
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<tr>
<td>96057</td>
<td>13212</td>
<td>12297</td>
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<td>95054</td>
<td>9049</td>
<td>8694</td>
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<tr>
<td>95092</td>
<td>12159</td>
<td>13394</td>
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Note: NBB: Brain bank number
clear effect of the PMD is observed in paraffin tissue (Lucassen et al. 1995). These data underline the importance of PMD and FD as matching factors when comparing human brain tissue using quantitative in situ hybridization (Ravid et al. 1992; Barton et al. 1993). In our studies, no significant correlation was found between the hybridization signal and the postmortem delay (ranging from 2h 30min to 85h 45 min) and FD (ranging from 27-130 days) in AVP mRNA levels in the human SCN (Chapter 3,4).

The decreased AVP mRNA levels in the SCN of AD patients correspond well with previous reports showing a major reduction in the number of neurons expressing AVP peptide in the SCN (Chapter 5). The changes in messenger and peptide do not always occur in the same direction. For instance, a discrepancy was found between these two measures in the SCN of depressed patients, where an increased number of neurons expressing AVP peptide was accompanied by decreased AVP mRNA levels. They hypothesized that there was not only a lower AVP synthesis, but also a decreased transport and/or release of AVP in depressed patients (Zhou, submitted 2000). It is thus necessary to perform not only peptide studies, but also to extend them with in situ observations in order to make a correct functional interpretation possible.

It is interesting to mention that animal studies have shown that the peak AVP peptide level in the SCN lags 20 h behind the peak for AVP mRNA. Due to the limited number of cases (5 subjects) who were younger than 50 years in our study, we were not able to perform a statistical analysis on the relationship between AVP mRNA and the number of neurons expressing AVP peptide in the SCN. However, the few data available showed the highest AVP mRNA levels in the SCN around 7 pm (chapter 5,6), whereas the rise in number of neurons expressing AVP peptide took place around 7 am (Hofman et al. 1994), indicating a clock period of 12 hours. Many more AVP mRNA measurements have to be performed to substantiate this estimation.
II CSF melatonin levels in relation to early stages of NFT and NP formation, aging and Alzheimer's disease

Changes in the circadian organization of neuroendocrine functions reflect alterations occurring in the brains of old people and may hence help to enlighten the pathophysiology of the aging brain. Melatonin is a hormone that plays not only a major role in the regulation of circadian rhythms, but may also exert neuroprotective effects in AD. These effects are attributed to its antioxidative and anti-β-amyloid toxicity actions as shown in vitro (Pappolla et al. 1997; Pappolla et al. 1998; Cassone et al. 1986; Mecacci et al. 1994; Richardson et al. 1996; Markesbery, 1997). It has also been proposed that the high levels of ventricular fluid melatonin in intact controls may protect the brain against hydroxyl radical damage and β-amyloid toxicity, especially as far as periventricular metabolically active neural tissue is concerned (Parker et al. 1994), and that this protective mechanism would fail in AD. Reliable well-controlled in vivo experiments on the presumed anti-aging effects of melatonin should, however, still be performed. We found that the CSF melatonin levels were 5-fold lower in AD patients than in age-matched controls, which agrees with the presumption that melatonin may indeed be involved in the pathogenesis of AD. It has been proposed in several studies that the gradual loss of melatonin levels in advanced age (Poeggele et al. 1993; Reiter et al. 1994), and even more so in AD patients (chapter 2,3), might contribute to the AD process (Maurizi, 1997). Our observation that there is a negative relationship between CSF melatonin levels and Braak stages (Chapter 3) seem to support this possibility. The same holds for our observation that the MBSC scores of the early AD changes for the temporal cortex are correlated to diminished CSF melatonin levels. The temporal cortex is the brain area where the AD process starts (Braak & Braak, 1996). Those controls that did not have any NFT or NP had much higher CSF melatonin levels than the controls that had a few NFTs or NPs in the temporal cortex. In the other cortical areas, i.e. frontal, parietal and occipital, we did not find such a relationship between decreased CSF melatonin levels and the occurrence of neuropathological changes. This finding could not be explained by a difference in the age, sex or clock time of death between the two groups (chapter 3). Our data, therefore, point to the possibility that the temporal cortex is the most vulnerable cortical area to the decreased CSF melatonin levels in the AD process (chapter 3). An interesting finding of the present study is that CSF-melatonin levels from ApoE-ε 3/4 genotype patients were significantly higher than those from ApoE 4/4 genotype (chapter 2). Since ApoE-ε4 is a major risk factor for AD this relation again suggests the involvement of melatonin in the pathogenesis of AD (chapter 2). On the basis of our findings (chapter 2,3) we hypothesize that the decrease of CSF melatonin levels may be an early event in the development of AD, occurring even before the clinical symptoms begin to show. To test this hypothesis in future studies we will follow saliva melatonin during the development of AD symptoms and determine the content of pineal monoamines and their metabolites in the subjects who
CHAPTER 8

have the earliest AD neuropathology (Braak stage 1 and 2) and in those that have no AD neuropathology (Braak stage 0).

The mechanisms leading to the lower levels of melatonin in aging and AD are not yet understood. Greenberg & Weiss showed that the β-adrenergic receptors in the pinealocyte membranes of aged rodents were diminished and less responsive to norepinephrine (Greenberg & Weiss, 1978). It has also been shown that, in older rats, the activity of N-acetyl-L-tyrosine (NAT), the key enzyme responsible for the synthesis of melatonin, decreases dramatically in the pineal in parallel with a decline of rat serum melatonin levels, independent of the circadian stage (Selmaoui & Touitou, 1999). Few data are available on the human pineal gland in aging and AD.

The observed decline with age of melatonin in the CSF levels (chapter 2) agrees with previous reports (Sack et al. 1986; Iguichi et al. 1982; Waldhauser et al. 1988; Skene et al. 1990). The decreased AVP mRNA expression in the SCN was also found in controls older than 80 years (chapter 5), suggesting that the changes in the SCN and pineal are related. In a large sample of human pineal glands (2700), a general selective senile atrophy of the pineal was not found (Gusek, 1983). There were also no obvious age-related ultrastructural changes in the human pineal gland (Hasegawa et al. 1990). Neither the structural changes of the pineal, such as the increased amount of calcifications, nor the variations of melatonin clearance seem to play an important causal role in the decrease of plasma melatonin levels in elderly subjects (Dori et al. 1994; Ferrari et al. 1995; Grad & Rozencwaig, 1993; Magri et al. 1997a). In addition, there is no evidence of the presence of neurofibrillary tangles or beta/A4 amyloid deposition in pinealocytes in the case of AD or in old controls (Pardo et al. 1990). It is believed, therefore, that the degeneration of noradrenergic innervation of the human pineal gland may be responsible for the decline of melatonin production (Jengeleski et al. 1989). However, it should be mentioned that in that study the dopamine β-hydroxylase immunoreactive fibers, rather than noradrenaline itself, were found to be abnormal. Since in the Jengeleski study (1989) only 3 controls, 3 old subjects and 3 AD patients were investigated (Jengeleski et al. 1989) and no direct measurement of noradrenaline and its metabolism were performed, a systematic study on the changes of pineal innervation in aging and AD is necessary.
III Free melatonin and aging

The finding of a significant negative correlation between low melatonin levels and the earliest neuropathological changes in aged controls (chapter 3) are of particular interest, because this may provide information about the very first stages of the disease that we have so far not been able to monitor in any other way. A relevant question is whether the measurement of total melatonin or free melatonin or the free/total melatonin ratio, which are regarded as sensitive indices of aging, have to be used for future research. Melatonin concentrations were reported to be markedly decreased in elderly subjects and AD patients in plasma and CSF (Dori et al. 1994; Ferrari et al. 1995; Grad & Rozencwaig, 1993; Magri et al. 1997a), chapters 2,3). However, a feature of almost all of these assays is that they have measured the total amount of melatonin (Kennaway & Voultsios, 1998). In the last study melatonin was found to be bound to albumin (Cardinali et al. 1972). A study by Morin et al. confirmed melatonin binding to albumin and also provided evidence for melatonin binding to the acute phase protein, α₁-acidglycoprotein (Morin et al. 1997). Unbound melatonin represents approximately 23% of the total plasma concentration. Saliva melatonin levels reflect the circulating free hormone, as this fluid is devoid of albumin and globulins (Kennaway & Voultsios, 1998). In other endocrine systems, the level of plasma binding of hormones profoundly affects the biological activity of the hormones. It is, for instance, quite possible that free, rather than bound, plasma cortisol can best reveal age effects in cross-sectional studies. However, an argument against an important physiological role of plasma binding of melatonin is its very low affinity (Kennaway & Voultsios, 1998). Therefore, binding of melatonin to proteins may have little effect in buffering transfer of melatonin across membranes or preventing binding to the high affinity melatonin receptors. Albumin-bound melatonin was also observed to cross the blood-brain barrier in rats (Pardridge & Mietus, 1980). Since the major protein responsible for binding melatonin in blood is albumin, and it is established that the concentration of this protein is lower in elderly subjects than in younger subjects (Davis et al. 1985; Viani et al. 1992), elderly subjects are expected to have less plasma-bound melatonin and higher free melatonin level than young people. Apart from aging, a number of other physiological conditions may lead to alterations in the levels of melatonin binding proteins and change the amount of free melatonin levels in the circulation. This makes further studies on the free/total melatonin ratio within and across age groups in saliva and plasma necessary.

In the near future we want to investigate saliva melatonin levels during the development of AD symptoms. As a first step we investigated the changes of saliva melatonin levels in aging. Alterations in the circadian rhythms of saliva melatonin occurred early in life, around 40 years of age (chapter 4). This is of particular interest, since the circadian fluctuations in the number of AVP neurons in the human SCN diminished in subjects older than 50 years (Hofman & Swaab, 1994). Moreover, a number of middle-age-
related circadian rhythm changes have been reported in the human, i.e. a dramatic decrease in the number of VIP neurons in the SCN in middle-aged male subjects (Zhou et al. 1995). Alterations in the human circadian time system thus already begin in middle-age, much earlier than generally thought. It will be interesting to further investigate the relation between free melatonin levels and sleep impairment or cognitive function.
IV AVP mRNA changes of the SCN in aging, Alzheimer’s disease and in glucocorticoid- treated patients

Several studies have shown the presence of circadian rhythm disturbances in aging, AD and corticosteroid-exposed patients (Mirmiran et al. 1992; Hofman & Swaab, 1994; Liu et al. 1999; Moser et al. 1996; Wolkowitz, 1994; Gift et al. 1989; Braunig et al. 1989). The present thesis supports the idea that these behavioral disturbances most probably have their basis in a decreased activity of the SCN. We found a strong decrease of AVP mRNA expression in the SCN of the AD patients (chapter 5). The total amount of AVP mRNA in the SCN was three times lower in AD patients than that in age and clock time of death matched controls, and the total number of profiles that expressed AVP mRNA in SCN in AD patients was only 40% of that of controls. The low amount of AVP mRNA was also found in controls older than 80 years. It is interesting to note that the day-night fluctuations in the amount of AVP mRNA were only observed in controls younger than 80 years. The decreased AVP mRNA levels in the SCN in AD patients corresponds well with previous reports showing a major reduction in AVP peptide levels of the SCN (Swaab et al. 1985b; Stopa et al. 1999).

The effect of corticosteroids on sleep architecture is of clinical significance in patients with chronic diseases (Moser et al. 1996). In order to see whether increased glucocorticoid levels may be a factor involved in the mechanism of circadian rhythm disturbance in old and demented people, we investigated the expression of AVP mRNA in the SCN of corticosteroid-treated patients whose average age was 53 years. We found that the SCN AVP mRNA expression was lower in the patients who were treated with exogenous corticosteroids or had a very high level of plasma cortisone because of a tumor. Also the total number of cell profiles that expressed AVP mRNA in the SCN was decreased in glucocorticoid-exposed patients (chapter 6). Several animal studies showed that an increased release of AVP from SCN terminals during the light period coincided with low levels of circulating corticosterone at that time of the day (Reppert et al. 1981; Kalsbeek et al. 1995). A similar relationship was observed in aging humans and Alzheimer patients, whose increased basal levels of cortisol (Dodt et al. 1994; Van Cauter et al. 1996) go together with a pronounced decline of AVP activity in the SCN (chapter 5). Only a limited number of animal studies have shown that adrenal steroids affect the AVP content of the SCN (Isobe & Isobe, 1998). Adrenalectomy as well as treatment of the rats with dexamethasone clearly demonstrate that the expression of AVP mRNA in the SCN is susceptible to alterations in circulating levels of glucocorticoids (Larsen et al. 1994). Moreover, we found that this suppressive effect of corticosteroid on AVP mRNA expression in the human SCN is rapid and reversible (chapter 6), depending on a number of factors, including the brain structure, the chemical nature of the neurons and the medicine involved. Concerning the latter factors, the type of the preparation used, dose, duration of the treatment and individual variation seemed of importance (Erkut et al. 1998, chapter 6).
In order to see whether increased glucocorticoid levels may affect circadian rhythms in old people as well as in young healthy subjects, seven female students, screened for a variety of health and life style factors, were studied 21 days by actigraphy before and during a period of stress. During the period of examinations a significant increase in perceived stress scores was observed. Academic stress appeared to increase the fragmentation of circadian rest-activity rhythms and to induce disruption of the sleep-wake rhythm (chapter 7).

In the present thesis we hypothesize that since vasopressin from the SCN inhibits the HPA-axis (Kalsbeek et al. 1996b; Kalsbeek et al. 1996a, chapter 5) and corticosteroid inhibits the vasopressin production of SCN neurons (chapter 6), the vasopressin neurons of the SCN seem to be incorporated in the feedback system of the HPA-axis. The decreased CSF melatonin levels we observed (chapter 2,3) coincide with a general disturbance of circadian rhythms in AD, e.g., in sleep-wake, body temperature and rest-activity (Van Someren et al. 1993) and with the degeneration of the SCN in aging and AD (chapter 5). It has been proposed that stimulation of the circadian system may at least partly restore these rhythms and have important therapeutic consequences for AD patients and elderly people (Van Someren et al. 1997). Such stimulation may even be beneficial for cortisol-treated patients. This notion is supported by increasing evidence showing that bright light, an interference presumed to stimulate the SCN directly, is a powerful synchronizer that can normalize the disturbed circadian rhythm and sleep, and can also substantially reduce behavioral disorders in AD (Campbell et al. 1988; Van Someren et al. 1996). In addition to light, non-pharmacological manipulation of circadian rhythms by means of various external stimuli, e.g. transcutaneous electrical nerve stimulation (Scherder et al. 2000; Scherder et al. 1992), also appears to be effective in improving sleep and cognitive functioning of elderly people and AD patients (Van Someren et al. 1997) and circadian rhythms (Scherder et al. 2000). It would be of considerable interest to study the SCN and pineal of AD patients that have been exposed to light and come to autopsy in order to see whether the disease-related changes in the circadian system we reported are reversible.

In the present thesis, a dramatic decrease in the CSF melatonin levels was found in old control subjects and even more so in AD patients. The diminished CSF melatonin levels already showed up in the aged controls with very early AD changes in the temporal cortex. We argued that also the alterations in the circadian rhythms of saliva melatonin occur very early, around 40 years of age. In addition we did not only find a strong decrease of AVP mRNA expression, but also much lower profiles expressing AVP mRNA in the SCN in aging, corticosteroid-treated patients and AD patients. These findings suggest that degeneration of the SCN-pineal complex could well be the neural substrate for the disrupted circadian rhythms reported in elderly corticosteroid-exposed patients and AD patients.