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Deterioration and reactivation
of the biological clock in dementia

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KEEP IT BRIGHT
Deterioration and reactivation of the biological clock in dementia

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Chapter 1

Preface & outline

Preface & outline

The research described in the present thesis focuses on common problems in dementia which all put a high burden on the quality of life of both the demented patients themselves and their caregivers. These problems not only consist of cognitive disabilities, but also of disturbances in behaviour, mood and the circadian regulation of sleep, hormones and temperature. For example, disturbed sleep-wake rhythms increase the odds for institutionalization within a year by a factor 10 [1]. It is often insufficiently recognized that the latter disturbances play an important role in patient management. Though less severe, these disturbances in dementia are also found in normal aging. The functionality of the circadian timing system (CTS) deteriorates with aging and is even more disturbed in demented elderly [2, 3]. Elderly suffer significantly more from sleep disturbances than younger people do and over half of the elderly population experiences at least one sleep complaint [4].

Deterioration and reactivation: "Use it or lose it"

The general hypothesis studied in this thesis is that deterioration of the suprachiasmatic nucleus (SCN), i.e. the biological clock, is for a large part responsible for disturbed functionality of the CTS, and contributes to disturbances in sleep, mood, behaviour and cognition in demented elderly. Moreover, we hypothesize that reactivation of the biological clock by the proper stimuli will improve the functionality of the biological clock, or prevent its deterioration. According to this hypothesis, enhanced exposure to the stimuli to which the biological clock is most sensitive, i.e. bright light and melatonin, should be a rational method to ameliorate sleep disturbances and to improve mood, behavioural and cognitive disturbances, at least as far as they are influenced by the CTS.

Chapter 2 reviews the CTS in old age and to what extent functional plasticity is maintained. It provides an overview of the different components of the CTS, the innervation and output of the SCN, and the occurring changes with aging. In this chapter the functional implications of weak and disturbed circadian rhythms regarding health and vital functions, mood and cognitive performance are discussed. The second part of this chapter focuses on the role of light exposure in the CTS, on the lack of exposure many elderly people suffer from, and on the effect of additional light exposure. It is demonstrated that a weakened circadian rhythm expression in elderly has negative health consequences. It, moreover, illustrates that the CTS shows a high degree of plasticity and responsiveness to supplementation of stimuli that are normally involved in the synchronization of endogenous and exogenous rhythms, most specifically to environmental light. However, this responsiveness has so far only been demonstrated in short term studies.

Chapter 3 reviews a major output of the CTS, the pineal gland and its principal hormone melatonin in relation to aging. A description is given of the pathway innervating the pineal gland and the production of melatonin from its precursors tryptophan and serotonin. The review shows that the 24-hour amplitude in melatonin secretion slowly decreases with age, and possibly even more so in demented elderly and elderly who have insufficient exposure to environmental bright light. Since melatonin, like light, provides a feedback input signal to the SCN, the decreased melatonin amplitude may further contribute to the lack of stimuli that regulate its activity. Clinical studies on supplementation of melatonin in elderly subjects again suggest a high degree of plasticity of the CTS. However, as with light, this responsiveness has so far only been demonstrated in short term studies.

Together, these two chapters provide a frame of reference and scientific basis for

the hypothesis that long-term enhancement of the often rather weak input to the CTS could enhance its functionality, and thereby improve nocturnal sleep and daytime functions. To which extent the *combined* supplementation of light and melatonin improves efficacy has not been studied before, nor whether efficacy is maintained on the long term. In addition, the possible role of such supplementation in the prevention of circadian rhythm disturbances has not been yet studied before. These questions have been addressed in the double-blind placebo-controlled randomized follow-up study described in chapters 7 and 8 of this thesis.

This thesis follows in a range of previous research projects based on the concept "Use it or lose it" in relation to the aging brain and dementia [5-11]. The concept is based on the hypothesis that neurons deprived from stimuli are at higher risk of degeneration and loss of function. Stimulation with the appropriate input is hypothesized to restore this process and increase functionality of the neurons. In case of the CTS, environmental light and melatonin are respectively external and internal stimuli for the suprachiasmatic nucleus that are essential for daily synchronization and optimal functionality. Elderly persons and, even more so, demented elderly, are exposed to less environmental light [12, 13]. Also the peak-level of melatonin is decreased in elderly, resulting in a diminished amplitude of the melatonin rhythm [14-22]. In line with the above mentioned hypothesis we expected that by increasing the daily light input and nighttime melatonin levels the highly prevalent disturbances in sleep-wake rhythms might be improved. Witting and colleagues showed that in aged rats the age-related disturbances in the rest-activity rhythm were prevented by bright light [11], while Lucassen showed that bright light prevented the decrease in vasopressin expression in the SCN in aged rats[5]. Van Someren et al. showed that in patients suffering from severe Alzheimer dementia (AD), disturbed rest-activity rhythms could be reversed by 4 weeks of indirect bright light [8]. The double-blind placebo-controlled randomized follow-up study described in the present thesis aimed to answer the question whether long term application of bright light in elderly residents of group care facilities, of which the majority was an earlier stage of Alzheimer's disease, might not only improve sleep-wake rhythm disturbances, but also prevent their development or attenuate their progression. A second question was whether supplementation with only one "Zeitgeber", light or melatonin, or the combination of two "Zeitgebers" shows different effects in the treatment of sleep-wake rhythm disturbances.

The main purpose of this randomized, placebo-controlled follow-up study was to investigate whether long–term treatment with bright light and/or melatonin - effectuating an enhanced input to the CTS - might counteract disturbed rhythms of sleep, hormone levels and temperature. In addition, we evaluated the possible secondary effects of the treatment on disturbed cognition, mood and behaviour. This evaluation was accomplished not only by calculating correlations but also by investigating whether treatment effects on cognition, mood and behaviour appeared to be mediated by treatment effects on affected circadian rhythm parameters.

Circadian timing system; relation with mood and behaviour

Circadian rhythm disturbances have been proposed to contribute not only to sleep problems, but also to behavioural disturbances and mood disorders [23-26]. Chronobiological theories of depression are based on three sets of data [24]. The first set consists of classical clinical phenomena, such as diurnal variation in depressive state, early morning awakening and seasonal modulation of the onset of mood disorders. The second

consists of the antidepressant effect of manipulations of the sleep-wake cycle and exposure to light. Furthermore, many circadian rhythms measured in depressed patients are abnormal, e.g. a diminished amplitude of the body temperature [25, 27, 28]. Recent observations show that even a primary origin of circadian rhythm and mood disturbances may be located in the central pacemaker, because polymorphisms in the clock gene NPAS2 are found to be associated with seasonal affective disorder[29].

The present thesis includes three studies, each with a different focus on the relation between mood and circadian timing system.

Chapter 4 focuses on the functional neurobiology of the central pacemaker in depression. Data of a post-mortem study are presented, comparing the expression of vasopressin in the SCN between depressed and non-depressed subjects. This study was based on the hypothesis that the functional ability of the SCN in maintaining normal biological rhythms might be diminished in depression. Vasopressin was studied because it is the major output neuropeptide of the SCN that reflects the circadian function of the SCN.

Chapter 6 focuses on the relation between mood and circadian rhythms at the behavioural level. Data are presented on the relation between the rest-activity rhythm on the one hand and disturbances in mood and social interaction on the other. Nonparametric variables were calculated from actigraphic data (for detailed description of the assessments, see below: General introduction to the clinical trial). Two methods for quantification of activity rhythms were applied. One of the methods assumes that the rest-activity rhythm has a periodicity of 24 hours and that two distinct periods of activity are expected to be observed during the course of a single 24-hour period: a sleep period and a wakefulness period. The method calculates two indices: the wake-active-index (WAI) and the sleep-inactive-index (SII) [30]. The second method for quantification of activity rhythms calculates the interdaily stability (IS), the intradaily variability (IV) and the relative amplitude (RA)[8]. A decrease in IS and RA and an increase in IV are typical features observed in demented elderly and are known to be sensitive for improvement by light therapy [8]. We investigated the association between these different rest-activity parameters with disturbances in mood and social interactions, but also with cognition, disease progress and the competence to perform daily activities. This study was based on baseline data from the clinical trial with light and melatonin that is extensively described in the General Introduction to the clinical trial and the Chapters 7 and 8.

Whereas Chapters 4 and 6 present correlative studies, Chapter 8 goes one step further. It describes an experimental study on the effect of chronobiological interventions on mood and other non-cognitive symptoms in mostly demented elderly residents of group care facilities. Light therapy is an effective treatment in itself for winter depression [31, 32] and, to a lesser extent, in non-seasonal depression [33] and may even play a more important role in combination with antidepressant drugs, where it seems to have an synergistic effect [34, 35]. Since both demented and non-demented elderly show blunted circadian rhythms as well as a larger prevalence of depression [36, 37], we hypothesized that depression in elderly residents of group care facilities could be worsened or even partly caused by disturbances in the circadian timing system - either in the SCN itself or in its input. The input to the SCN was augmented by increasing environmental light or by the intake of melatonin.

Circadian timing system and cognition in dementia

More and more experimental studies reported on the role of sleep in cognitive functioning, especially memory, in healthy young adults [38-40]. Also in AD, a relationship was found between the sleep-

wake pattern and cognitive functioning [41]. Particularly night-time wakefulness and REM sleep consistently predicted variance in cognitive and functional status measures.

Demented elderly by definition suffer from cognitive disturbances which are progressive and disabling. We hypothesized that poor sleep-wake rhythms may aggravate cognitive disabilities and that this aggravation is, at least partly, reversible when sleep-wake rhythm disturbances are successfully treated by chronobiological interventions. Some studies already showed promising results in this direction. Yamadera et al.[42] studied the effect of bright light on cognition in a group of 27 AD patients and found an improvement of both cognition, as measured by the MMSE, and circadian rhythmicity after 4 weeks of bright light. Other studies have been carried out on the effect of melatonin on cognition in AD, which found a positive effect of melatonin treatment on cognition [43-45]. However, as was also concluded by a recent Cochrane review, the short period of the studies and methodological deficiencies do not yet support the use of melatonin for treatment of cognitive impairment associated with dementia and AD [46]. In our double-blind placebo-controlled randomized long-term follow-up study on the effect of light and/or melatonin, 189 subjects were followed for an average duration of 15 months, up to 3.5 years for a gradually more and more limited number of participants. With this long-term application of the intervention and long-term follow up with assessments every 6 months, we hoped to find more evidence for a chronobiological approach that would also affect cognitive deterioration in mostly demented elderly residents of group care facilities. In Chapter 7, the effects of the intervention on sleep and cognition are described. Because a simultaneously found positive effect on both sleep and cognition would not necessarily mean that the effect on cognition is due to an improvement in the sleep-wake rhythm, we included multilevel mediation analyses [47] in order to investigate the possibility that treatment effects on cognitive performance were in part mediated by their effects on the sleep-wake rhythm.

General description of the double-blind placebo controlled randomized

Follow-up study

Our study was performed between June 1999 and April 2004 in twelve different homes for the elderly with a total number of 189 subjects. Analyses of baseline data and the results of the follow-up study are described in the Chapters 5, 6, 7 and 8. Since each Chapter describes its respective part and topic of the study, a general overview of the study design will be outlined here.

Study design

A total number of 12 homes for the elderly in different places in the Netherlands participated in the study. Of each home, we invited the residents of the assisted care (group care) facility to participate in the study. These facilities provide assistance in activities of daily living, and the majority of the residents are demented. A minority of the residents stayed in the facilities for social or physical reasons. We did not want to exclude these residents from the study because deterioration of the CTS is a general problem in aging, not exclusive for demented elderly. Also non-demented institutionalized elderly are deprived from outdoor light exposure and it can thus be expected that this group of residents might benefit from the treatment as well. Clinical diagnosis of dementia was included as a covariate in the statistical analyses to evaluate possible modification of the outcome measure effects.

All family members signed informed consent after information was given about background and study design. The study was approved by the Medical Ethical Committee

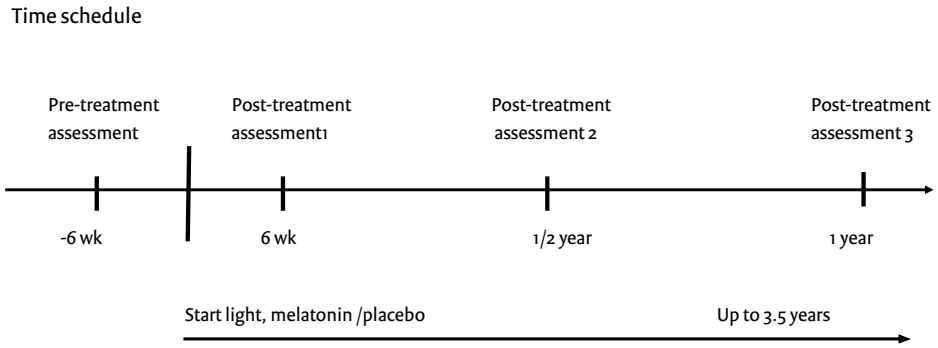


Figure 1
Schematic overview of the time schedule. A pre-treatment assessment was performed 6 weeks before the start with light, melatonin or placebo treatment, the first post-treatment assessment was performed 6 weeks after the start of the treatment, the second until the eight post-treatment assessments were performed with half-yearly intervals after the start of the treatment.

of hospital De Gelderse Vallei (Ede, The Netherlands). For assessment of the ApoE genotype a separate approval was obtained from the Medical Ethical Committee of the VU University Medical Center (VUmc, Amsterdam, The Netherlands).

Although we originally aimed to start in all the homes for the elderly around the same time and follow them up for 3.5 years, this appeared not to be possible due to logistical problems. We therefore decided to start first in 8 homes for the elderly and at a later time point in the other 4 homes. The first eight homes for the elderly started in the period of June - August 1999. Four of them stayed in the study for the entire period, which ended April 2003. This resulted in a 3.5 year follow-up with half-yearly assessments. One home stopped participation after 6 months because of rebuilding of the facility. Two homes quitted the study after 1.5 year because the nursing staff had problems continuing the study due to lack of time to fill out the questionnaires. One of them was in the placebo light group and one in the bright light group. One home quitted the study after 8 assessments (3 years follow up) because of moving into another room.

Two homes started in May 2000 and remained in the study until the end of the study in April 2003, resulting in 2.5 year follow-up. Two homes started in January 2001 and were followed up for 2 consecutive years of half yearly assessments until the end of the study in April 2003.

Within the homes, 129 subjects participated in the study from the beginning. They were already living in the participating homes for the elderly at the moment the study started and participated in the baseline assessment. Another 60 subjects started later, i.e. as of the moment they came to live in the participating home for the elderly where the study was already going on. We visited the homes for the elderly every half year to obtain treatment-effect assessments. During these visits, all participating subjects living in the respective homes were assessed. Due to logistical reasons it was not possible to pay an extra visit every time a new inhabitant came to live in the assisted care facility of a home for the elderly. All new participants were therefore tested for the first time at the subsequent treatment effect assessments of the participants who were already in the study. The first assessment of these new participants was therefore not a baseline assessment, but a first effect assessment; their data were entered in the dataset accordingly. Although their baseline measurement was

missing, the application of multilevel regression analyses still allowed their data to be included for the evaluation of group differences and changes over time in the outcome variables (see below: statistical analysis). End points for participation in the study were nursing home placement or passing away of a subject.

A baseline assessment of all the outcome variables was performed 6 weeks before the lights were placed in the common living room and the administration of melatonin. Six weeks after the start of the treatment a short-term assessment was performed and thereafter the assessments were performed every half year up to a maximum of 8 post-treatment assessments. Figure 1 shows the time schedule in a schematic way. Figure 2 A and B show the cumulative frequency distribution and a flow chart of all the subjects who entered the study and the reason for dropout at each assessment point per group.

Light & Melatonin supplementation and randomization

The 12 assisted care facilities were assigned to either the active light condition or placebo light condition by stratified randomization. We chose for stratified randomization because differences in the quality of the nursing home environment were expected to influence the outcome. All care facilities were rated on the Therapeutic Environment Screening Scale (TESS)[48]. The TESS rating was used for the stratified randomization of the light treatment condition. In the active bright light condition, ceiling mounted light (Philips, TLD 840 and 940 fluorescent tubes, Eindhoven, The Netherlands) provided about 1000 lux in gaze direction. In the placebo condition similar fixtures provided only about 350 lux in gaze direction. The placebo condition was created by mounting identical fixtures with fewer tubes at less favourable places and installing full band stop filters resulting in light intensities not different from the pre-treatment situation. The light intensities of the two conditions in both the pre- and post treatment situation are presented in Figure 3. The subjects, caregivers, family and neuropsychologist were blind to the light condition. In order to verify whether we thus had effectively implemented a placebo condition, caregivers were requested to fill out a brief questionnaire half-yearly to indicate whether they believed their location was assigned to the more effective light condition (yes, no, no idea). There was no significant difference between 184 ratings obtained from 89 caregivers over the treatment period on an "illumination pleasantness" visual analogue scale ($p=0.47$) and on the odds of a confirmative answer to the question whether they thought their facility had effective light ($p=0.62$).

All subjects in both the active and placebo light condition were furthermore randomly assigned to receive either a tablet containing melatonin (2.5 mg, intermediate release, Terafarm, Katwijk, The Netherlands) or a placebo tablet of the same size and colour, administered daily approximately 1 hour before bedtime. Randomization was performed using the Microsoft Excel 'rand()' function.

After randomization, the number of subjects in each of the 4 treatment groups was as follows: 49 subjects received only bright light, 46 only melatonin, 49 received both light and melatonin and 45 were assigned to the double placebo group.

Outcome measures

The outcome variables could roughly be divided into two groups:

1) Circadian and sleep variables and
2) Neuropsychological variables.

1) Circadian and sleep variables

To study rest-activity rhythms and sleep we used the actiwatch (Cambridge Neurotechnology, CNT, Cambridge, UK), a wrist worn device that measures activity and light intensity. On every assessment occasion, estimates of sleep duration, sleep efficiency (percentage of being asleep), sleep latency and average duration of periods of nocturnal wakefulness were obtained from

14 ± 4 (minimum 3) days of actigraphy, analysed with the validated Sleep Analysis 2001 software (CNT) [49], using habitual bedtime and get-up time provided by the nursing staff on every occasion. The 24-hour rhythmic aspects of activity were quantified using non-parametric parameters [50].

Furthermore, the circadian melatonin and cortisol rhythms were assessed from saliva samples, taken at different time points over 24 hours (see figure 4). Another frequently used parameter to assess SCN functionality is the 24-hour temperature rhythm. We measured this parameter by assessing the tympanic temperature at the same time points when a saliva sample was taken. Tympanic temperature was chosen because of the easy applicability in demented elderly. Tympanic thermometry shows less variability then electronic oral measures and correlates significantly with rectal assessments of body temperature [51].

2) *Neuropsychological variables*

The Neuropsychological assessments were performed by three different neuropsychologists who were blind to the treatment condition. Ideally, all assessments should have been done by one and the same neuropsychologist to avoid differences between the different raters. Due to the large group of subjects this was no option. Therefore, the subjects were assessed as much as possible by the same rater in subsequent assessments.

We used the mini-mental state examination (MMSE) and the cognitive part of the Alzheimer's disease assessment scale (ADAS) to assess cognitive functioning.[52, 53] Both scales are widely applied for clinical use and research purposes [54-56]. Although a high compliance and completion was reached for the MMSE assessment, the ADAS very often had to be discontinued before completion because many of the subjects were not able to perform the long lasting test of almost one hour and, therefore, was not included in the analyses.

For the assessment of non-cognitive symptoms we used the following rating scales. Mood was assessed by the Cornell Scale for depression in demented elderly (CSDD)[57], the Philadelphia Geriatric Center Affect Rating Scale (PGCARS)[58] and the Philadelphia Geriatric Center Morale Scale (PGCMS)[59]. Behavioural disturbances were assessed by the Multi Observational Scale for Elderly Subjects (MOSES)[60], the Neuropsychiatric Inventory questionnaire (NPI-Q)[61, 62] and the Cohen-Mansfield Agitation Inventory (CMAI)[63, 64].

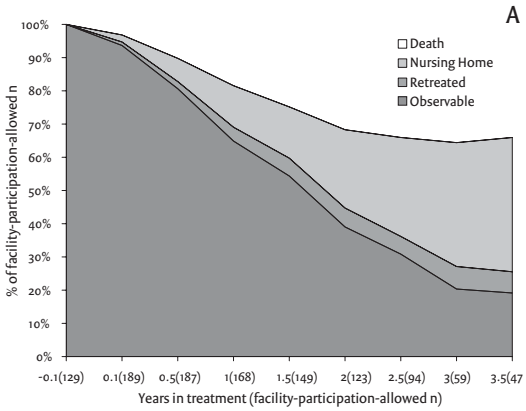
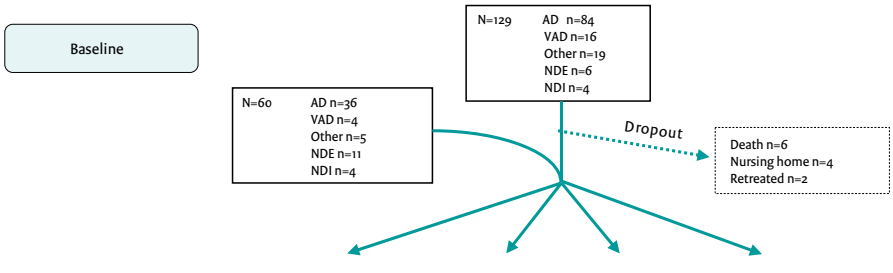


Figure 2

A. Cumulative frequency distribution of the percentage of subjects available for observation and the percentage lost due to retreat, nursing home placement or death. At each assessment point (horizontal axis) the percentage is expressed relative to the facility-participation allowed maximal number of subjects had everybody still been observable (given in brackets at the horizontal axis). Note that, of the observable cases, a percentage of assessments missed due to logistics, non-compliance or insufficient communication abilities (see text for numbers).

B. Flow chart of subjects included in the study. One hundred twenty nine subjects started with the pre-treatment assessment (AD = Alzheimer's disease, VAD = vascular dementia, other = other types of dementia, NDE = not demented, NDI = not diagnosed due to lack of data). Sixty subjects entered the study later (see text) and are included in the chart from post-treatment assessment 1 onwards, irrespective of the time relative to their facilities duration of participation. Reasons for dropout were death, nursing home placement, retreated informed consent or study end of the facility. The first post-treatment assessment 1 was 6 weeks after the start of the light and/or melatonin treatment, assessment 2 half a year after the start of light and/or melatonin and the subsequent assessments were done every next half year.

B



Post-treatment 1 6 wk	Light & Melatonin Total n=43	Light Total n=48	Melatonin Total n=46	Placebo Total n=40
	31 AD 6 VAD 3 Other 3 NDE 0 NDI	36 AD 5 VAD 6 Other 1 NDE 0 NDI	28 AD 3 VAD 6 Other 5 NDE 4 NDI	20 AD 4 VAD 7 Other 8 NDE 1 NDI

Post-treatment 2 6 months	N=150	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatonin	36		4	1	0	2
	Light	44		2	2	0	0
	Melatonin	39		2	3	1	1
	Placebo	31		5	3	1	0

Post-treatment 3 1 year	N=109	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatonin	26		1	5	0	4
	Light	34		0	3	0	7
	Melatonin	27		7	3	0	2
	Placebo	22		5	1	3	0

Post-treatment 4 1.5 years	N=81	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatoni n	21		1	1	0	3
	Light	24		4	2	0	4
	Melatonin	18		4	2	0	3
	Placebo	18		2	0	1	1

Post-treatment 5 2 years	N=48	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatoni n	12		4	3	0	2
	Light	17		3	2	0	2
	Melatonin	9		3	2	0	4
	Placebo	10		1	2	1	4

Post-treatment 6 2.5 years	N=29	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatoni n	9		2	0	0	1
	Light	14		1	1	0	1
	Melatonin	4		0	2	0	3
	Placebo	2		2	1	0	5

Post-treatment 7 3 years	N=12	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatoni n	3		0	2	0	4
	Light	5		0	2	0	7
	Melatonin	2		0	0	0	2
	Placebo	2		0	0	0	0

Post-treatment 8 3.5 years	N=9	Dropout	Death	Nursing home	Retreated	Study end
	Light & Melatoni n	3		0	0	0	0
	Light	3		1	0	0	1
	Melatonin	2		0	0	0	0
	Placebo	1		0	1	0	0

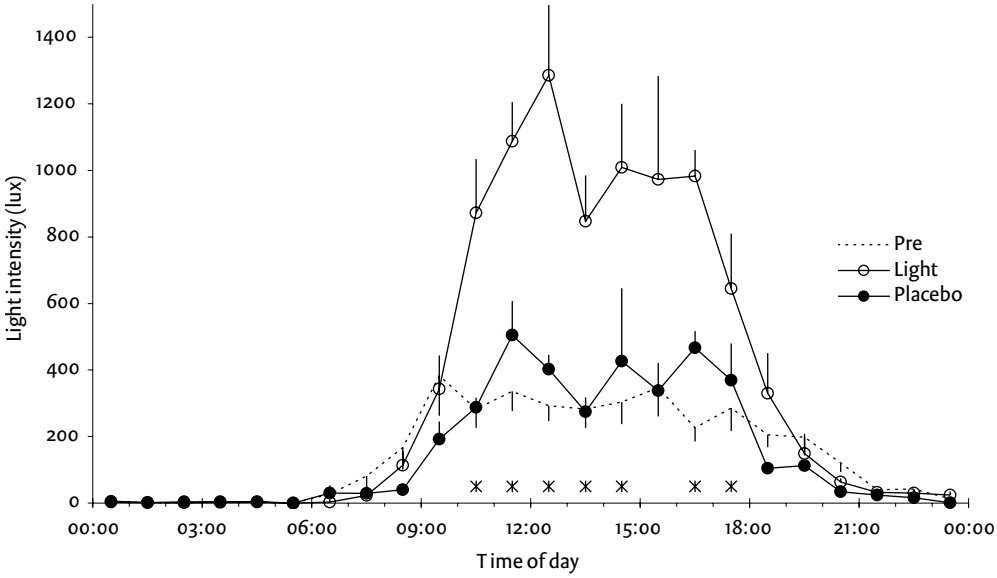


Figure 3
Average 24-hour light exposure profiles before (dashed line) and after installing the active (open circles) and placebo (closed circles) lights. Individual lux measurements (Mavolux Digital, Gossen, Nürnberg, Germany) were done at eye level in the direction of gaze, i.e. usually quantifying slightly downward or at best vertical illumination levels, which are considerably lower but more adequate than horizontal assessments directed towards the light sources. Assessments include observations made if subjects were not actually present in the common living room where the lights were installed, but in their own bedroom. Asterisks indicate the hours of significantly increased light intensity in the active condition ($p \leq 0.01$). None of the hours showed increased intensity in the placebo condition. Mean ± s.e.m. values were obtained from multilevel analysis of 3017 light measurements from 189 subjects in 12 facilities obtained over up to 3.5 years throughout the 24-hour day.

ApoE-genotype

The risk of ApoE genotype for Alzheimer's disease is known for more than a decade [65, 66]. The results of several studies give evidence for a role of ApoE genotype in the susceptibility for developing dementia even before there are enough clinical symptoms to reliably diagnose a patient demented. In addition, some studies have shown a differential effect of treatment according to ApoE genotype. One study showed that the effect of donepezil, an acetylcholine esterase inhibitor (AchE-I) used for the treatment of AD, on APP metabolism in platelets is different between ε4 and non ε4 carriers [67]. A study on the use of tacrine, another AchE-I, showed an accelerated cognitive decline in ε4 carriers as compared to non ε4 carriers[68]. However, a study on donepezil did not show an influence of ApoE-genotype on treatment effect [69]. Because of the possible influence of ApoE-genotype on cognitive deterioration and interaction with treatment outcome, we assessed ApoE-genotype from saliva samples in order to evaluate a modifying effect on treatment outcome.

Diagnosis of dementia and dementia subtypes

According to the Diagnostic and Statistical Manual of Mental Disorder 4th Edition (DSM-IV), dementia is a clinical syndrome characterized by the development of multiple cognitive deficits manifested by both memory impairment and one or more of the following cognitive disturbances: aphasia, apraxia, agnosia, disturbance in executive functioning [70]. Further classification is based on the possible cause of the

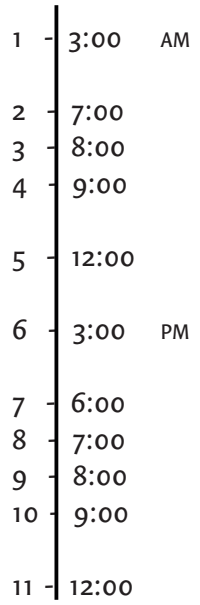


Figure 4
Sampling times at which saliva samples were taken and tympanic temperature was measured at each assessment.

dementia syndrome. For the diagnosis of possible or probable Alzheimer dementia (AD) the NINCDS-ADRDA criteria were used [71]. A definite diagnosis of AD can only be determined by post-mortem neuropathological examination of the subject showing neuritic plaques and neurofibrillary tangles[71]. Classification according to the DSM-IV of Vascular Dementia (VAD) requires evidence of cerebrovascular disease that is considered to be etiologically related to the dementia [70]. A definite diagnosis of VAD requires histopathological evidence of cerebrovascular disease and absence of neurofibrillary tangles and neuritic plaques exceeding those expected for age[72]. Because neuropathological examination was not included in the informed consent of the clinical trial, no definite diagnosis of AD or VAD could be made. Dementia due to other general medical conditions requires evidence of a general medical condition that is etiologically related to the dementia[70]. Substance induced persisting dementia is diagnosed if the symptoms persist beyond the usual duration of substance intoxication or withdrawal together with the evidence that the deficits are etiologically related to the persisting effects of substance use[70]. If the dementia has more than one aetiology, the disease is classified as dementia due to multiple etiologies[70]. When a demented subject does not meet the criteria for any of the specific types described above, the diagnosis should be classified as dementia not otherwise specified[70].

According to the available clinical data and history of the disease process, together with the clinical picture at entry of the study, we determined the clinical diagnosis based on the above mentioned criteria. In figure 2 the numbers of the different clinical diagnosis in our study are mentioned. The group of other types of dementia includes dementia due to other medical conditions, including Parkinson's disease and frontal type dementia; substance induced persisting dementia, dementia due to multiple etiologies and dementia not otherwise specified. In some cases we had too little information for a reliable diagnosis. These subjects were classified as not-diagnosed (NDI). Another few cases turned out to be not demented, but were in the group care facility for physical or social reasons. These subjects were classified as non-demented (NDE).

Statistical analyses

The first analyses involved all available observations of all subjects. Longitudinal hierarchical linear regression analyses [73] were applied to account for the three-level nested structure of the dataset, i.e. a variable number of observations nested within subjects, and subjects grouped in 12 facilities (MLwiN, Institute of Education, London, UK). These analyses were applied to account for the correlated data structure by including random intercepts (i.e. the observations on time i were nested within subjects j, which were once more nested in facilities k). This

approach resulted in separation of the residual error variances at the levels of facility, subject and observation.

Because previous studies on the effect of light and melatonin have been of limited duration, they did not allow for conclusions on whether light or melatonin treatment would remain efficacious when applied for a period of time than just a few weeks. Possible effects might as well fade out or, the reverse, grow slowly over months of treatment. Therefore, analyses were planned to evaluate both (1) treatment-effects that were immediate and of which the effect size did not change over time in treatment, as well as (2) time-by-treatment effects, i.e. with an effect size changing over time in treatment. Melatonin, light and their interaction were dummy coded in three variables indicating the presence of active treatment at any observation, i.e. 1 in case of active treatment and 0 for all observations prior to treatment onset and in case of placebo treatment. Given the longitudinal character of the dataset, 'time' was included in the model as a discrete factor. In the analyses, special attention was given to the fact that- especially after 1.5 years - many cases were lost from follow-up either due to noninformative reasons (discontinuation of participation of the facility) or to possibly informative causes. First, in order to obtain the most simple acceptable regression equation insensitive to a reduction in the follow-up time, we verified whether treatment effects obtained from analyses on the complete 3.5 year dataset were still present in a reduced dataset including only the first 1.5 year of follow-up. A second approach was to code missing data due to (1) death or nursing home placement, or (2) non-compliance or insufficient communication abilities, were considered to be informative and coded in two dummy variables to allow for inclusion in the regression analysis according to a pattern mixture model approach[74]. Thus, the initial full model multilevel regression equation fitted to the data was of the form:

$$\begin{aligned}\text{Outcome}_{ijk} = {} & \beta_{0ijk} + \beta_1 * \text{Light}_{ijk} + \beta_2 * \text{Melatonin}_{ijk} \\ & + \beta_3 * \text{Light} * \text{Melatonin}_{ijk} + \beta_4 * \text{Time}_{ijk} \\ & + + \beta_5 * \text{Time} * \text{Light}_{ijk} \\ & + \beta_6 * \text{Time} * \text{Melatonin}_{ijk} \\ & + \beta_7 \text{Time} * \text{Light} * \text{Melatonin}_{ijk} \\ & + \beta_8 * \text{MissingPattern1}_{ijk} \\ & + \beta_9 * \text{Time} * \text{MissingPattern1}_{ijk} \\ & + \beta_{10} * \text{MissingPattern2}_{ijk} \\ & + \beta_{11} * \text{Time} * \text{MissingPattern2}_{ijk}\end{aligned}$$

where each observation in time is denoted as *i*, each subject as *j*, and each facility as *k*. The betas provide the intercept (β_0) and effect estimates (β_1 to β_{11}). The regression equations were re-evaluated after each step of the stepwise exclusion of the least significant terms, of which the exclusion did not significantly increase the residual error of the equations according the −2loglikelihood ratio chi-square test with a two-tailed significance level set at 0.05 [50]. The resulting most simple acceptable regression equations thus included only variables with significant effect sizes. It was evaluated post-hoc whether level, time course or treatment effects were modified by Alzheimer's diagnosis (dummy coded 0-1) and by ApoE genotype (two dummy codes, respectively for the presence of at least one ApoE2 or at least one ApoE4 allele).

Statistical power

At the onset of the study it was estimated that subjects would, on average, remain in the protocol for 2.5 years, allowing for 6 follow-up assessments: one short-term and five half-yearly. Under the assumption of a within-subject correlation of r=0.50 and using the formulas provided by Twisk [73], 147 subjects would be necessary to attain, at an alpha of 0.05, a power of 0.80 to detect effect sizes of d=0.25 i.e. between the conventional definition of a small (0.20) to moderate (0.50) effect size. The absolute minimal aim was set at 140. Since new inhabitants, assigned to the special care facilities after onset of the study, were faced with the presence of the dedicated lighting systems, it was determined at the onset of the study that they were to be given

the possibility to participate, both because of ethical considerations and because of statistical power considerations: power and smallest detectable difference calculation outcomes vary with small deviations from estimated sample size, effect size or within-subject correlation. For these reasons, an unlimited number of subjects was allowed to enter the study until it was finished. The longest duration of data-acquisition in a single facility was set at 3.5 years after the start of the treatment.

References

1. Bianchetti, A. et al. Predictors of mortality and institutionalization in Alzheimer disease patients 1 year after discharge from an Alzheimer dementia unit. Dementia 6, 108-112 (1995).

2. Bliwise, D. L. Sleep in normal aging and dementia. Sleep 16, 40-81 (1993).

3. Van Someren, E. J. Circadian and sleep disturbances in the elderly. Exp Gerontol 35, 1229-37. (2000).

4. Foley, D. J. et al. Sleep complaints among elderly persons: an epidemiologic study of three communities. Sleep 18, 425-32. (1995).

5. Lucassen, P. J., Hofman, M. A. & Swaab, D. F. Increased light intensity prevents the age related loss of vasopressin- expressing neurons in the rat suprachiasmatic nucleus. Brain Res 693, 261-6 (1995).

6. Scherder, E. J., Van Someren, E. J. & Swaab, D. F. Transcutaneous electrical nerve stimulation (TENS) improves the rest- activity rhythm in midstage Alzheimer's disease. Behav Brain Res 101, 105-7 (1999).

7. Swaab, D. F. Brain aging and Alzheimer's disease, "wear and tear" versus "use it or lose it". Neurobiol Aging 12, 317-24 (1991).

8. Van Someren, E. J., Kessler, A., Mirmiran, M. & Swaab, D. F. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biol Psychiatry 41, 955-63 (1997).

9. Van Someren, E. J., Lijzenga, C., Mirmiran, M. & Swaab, D. F. Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. J Biol Rhythms 12, 146-56 (1997).

10. Van Someren, E. J. W., Scherder, E. J. A. & Swaab, D. F. in Alzheimer's Disease and Related Disorders (eds. Iqbal, K., Swaab, D. F., Winblad, B. & Wisniewski, H. M.) 771-779 (John Wiley & Sons Ltd, 1999).

11. Witting, W., Mirmiran, M., Bos, N. P. & Swaab, D. F. Effect of light intensity on diurnal sleep-wake distribution in young and old rats. Brain Res Bull 30, 157-62 (1993).

12. Campbell, S. S., Kripke, D. F., Gillin, J. C. & Hrubovcak, J. C. Exposure to light in healthy elderly subjects and Alzheimer's patients. Physiol Behav 42, 141-4 (1988).

13. Ancoli-Israel, S. et al. Variations in circadian rhythms of activity, sleep, and light exposure related to dementia in nursing-home patients. Sleep 20, 18-23 (1997).

14. Ferrari, E. et al. in Melatonin and the pineal gland - From basic science to clinical application (eds. Touitou, Y., Arendt, J. & Pevet, P.) 379-382 (Elsevier Science Publishers B.V., 1993).

15. Iguichi, H., Kato, K. I. & Ibayashi, H. Age-dependent reduction in serum melatonin concentrations in healthy human subjects. J Clin Endocrinol Metab 55, 27-9. (1982).

16. Mishima, K. et al. Melatonin secretion rhythm disorders in patients with senile dementia of Alzheimer's type with disturbed sleep-waking. Biol Psychiatry 45, 417-21 (1999).

17. Nair, N. P., Hariharasubramanian, N., Pilapil, C., Isaac, I. & Thavundayil, J. X. Plasma melatonin--an index of brain aging in humans? Biol Psychiatry 21, 141-50 (1986).

18. Sack, R. L., Lewy, A. J., Erb, D. L., Vollmer, W. M. & Singer, C. M. Human melatonin production decreases with age. Journal of Pineal Research 3, 379-388 (1986).

19. Touitou, Y. & Haus, E. Alterations with aging of the endocrine and neuroendocrine circadian system in humans. Chronobiology International 17, 369-390 (2000).

20. Zhdanova, I. V., Wurtman, R. J., Balcioglu, A., Kartashov, A. I. & Lynch, H. J. Endogenous Melatonin Levels and the Fate of Exogenous Melatonin: Age Effects. Journal of Gerontology 53A, B293-B298 (1998).

21. Rodenbeck, A., Huether, G., Ruther, E. & Hajak, G. Altered circadian melatonin secretion patterns in relation to sleep in patients with chronic sleep-wake rhythm disorders. J Pineal Res 25, 201-10. (1998).

22. Sharma, M. et al. Circadian rhythms of melatonin and cortisol in aging. Biol Psychiatry 25, 305-19 (1989).

23. Swaab, D. F., Van Someren, E. J., Zhou, J. N. & Hofman, M. A. Biological rhythms in the human life cycle and their relationship to functional changes in the suprachiasmatic nucleus. Prog Brain Res 111, 349-68 (1996).

24. Van den Hoofdakker, R. H. Chronobiological theories of nonseasonal affective disorders and their implications for treatment. J Biol Rhythms 9, 157-83 (1994).

25. Wirz-Justice, A. in Psychopharmacology: The Fourth Generation of Progress (eds. Bloom, F. E. & Kupfer, D. J.) 999-1017 (Raven Press, New York, 1995).

26. Volicer, L., Harper, D. G., Manning, B. C., Goldstein, R. & Satlin, A. Sundowning and circadian rhythms in Alzheimer's disease. Am J Psychiatry 158, 704-11 (2001).

27. van Londen, L. et al. Weak 24-h periodicity of body temperature and increased plasma vasopressin in melancholic depression. Eur Neuropsychopharmacol 11, 7-14 (2001).

28. Souetre, E. et al. Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality. Psychiatry Res 28, 263-78 (1989).

29. Johansson, C. et al. Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. Neuropsychopharmacology 28, 734-9 (2003).

30. Minors, D. et al. The difference between activity when in bed and out of bed. I. Healthy subjects and selected patients. Chronobiol Int 13, 27-34 (1996).

31. Eastman, C. I., Young, M. A., Fogg, L. F., Liu, L. & Meaden, P. M. Bright light treatment of winter depression: a placebo-controlled trial. Arch Gen Psychiatry 55, 883-9 (1998).

32. Lewy, A. J. et al. Morning vs evening light treatment of patients with winter depression. Arch Gen Psychiatry 55, 890-6 (1998).

33. Kripke, D. F. Light treatment for nonseasonal depression: speed, efficacy, and combined treatment. J Affect Disord 49, 109-17 (1998).

34. Benedetti, F. et al. Morning light treatment hastens the antidepressant effect of citalopram: a placebo-controlled trial. J Clin Psychiatry 64, 648-53 (2003).

35. Martiny, K. Adjunctive bright light in non-seasonal major depression. Acta Psychiatr Scand Suppl, 7-28 (2004).

36. Lee, H. B. & Lyketsos, C. G. Depression in Alzheimer's disease: heterogeneity and related issues. Biol Psychiatry 54, 353-62 (2003).

37. Purandare, N., Burns, A., Craig, S., Faragher, B. & Scott, K. Depressive symptoms in patients with Alzheimer's disease. Int J Geriatr Psychiatry 16, 960-4 (2001).

38. Walker, M. P. & Stickgold, R. Sleep-dependent learning and memory consolidation. Neuron 44, 121-33 (2004).

39. Drosopoulos, S., Wagner, U. & Born, J. Sleep enhances explicit recollection in recognition memory. Learn Mem 12, 44-51 (2005).

40. Wagner, U., Gais, S., Haider, H., Verleger, R. & Born, J. Sleep inspires insight. Nature 427, 352-5 (2004).

41. Moe, K. E., Vitiello, M. V., Larsen, L. H. & Prinz, P. N. Symposium: Cognitive processes and sleep disturbances: Sleep/wake patterns in Alzheimer's disease: relationships with cognition and function. J Sleep Res 4, 15-20 (1995).

42. Yamadera, H. et al. Effects of bright light on cognitive and sleep-wake (circadian) rhythm disturbances in Alzheimer-type dementia. Psychiatry Clin Neurosci 54, 352-3 (2000).

43. Asayama, K. et al. Double blind study of melatonin effects on the sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. J Nippon Med Sch 70, 334-41 (2003).

44. Serfaty, M., Kennell-Webb, S., Warner, J., Blizard, R. & Raven, P. Double blind randomised placebo controlled trial of low dose melatonin for sleep disorders in dementia. Int J Geriatr Psychiatry 17, 1120-7 (2002).

45. Singer, C. et al. A multicenter, placebo-controlled trial of melatonin for sleep disturbance in Alzheimer's disease. Sleep 26, 893-901 (2003).

46. Jansen, S. L., Forbes, D. A., Duncan, V. & Morgan, D. G. Melatonin for cognitive impairment. Cochrane Database Syst Rev, CD003802 (2006).

47. Krull, J. L. & MacKinnon, D. P. Multilevel modeling of individual and group level mediated effects. Multivariate Behavrioral Research 36, 249-277 (2001).

48. Sloane, P. & Mathew, L. The therapeutic environment screening scale. Am J Alzheimers Care Related Disorders Res 5, 22-26 (1990).

49. Kushida, C. A. et al. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. Sleep Med 2, 389-96 (2001).

50. Van Someren, E. J. et al. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int 16, 505-18 (1999).

51. Castle, S. C., Toledo, S. D., Daskal, L. & Norman, D. C. The Equivalency of Infrared Tympanic Membrane Thermometry with Standard Thermometry in Nursing Home Residents. J Am Geriatr Soc 40, 1212-1216 (1992).

52. Folstein, M. F., Folstein, S. E. & McHugh, P. R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12, 189-98 (1975).

53. Mohs, R. C. & Cohen, L. Alzheimer's Disease Assessment Scale (ADAS). Psychopharmacol Bull 24, 627-8 (1988).

54. Schmeidler, J., Mohs, R. C. & Aryan, M. Relationship of disease severity to decline on specific cognitive and functional measures in Alzheimer disease. Alzheimer Dis Assoc Disord 12, 146-51 (1998).

55. Wilcock, G. et al. A long-term comparison of galantamine and donepezil in the treatment of Alzheimer's disease. Drugs Aging 20, 777-89 (2003).

56. Seltzer, B. et al. Efficacy of donepezil in early-stage Alzheimer disease: a randomized placebo-controlled trial. Arch Neurol 61, 1852-6 (2004).

57. Alexopoulos, G. S., Abrams, R. C., Young, R. C. & Shamoian, C. A. Cornell Scale for Depression in Dementia. Biol Psychiatry 23, 271-84 (1988).

58. Lawton, M. P., Van Haitsma, K. & Klapper, J. Observed affect in nursing home residents with Alzheimer's disease. J Gerontol B Psychol Sci Soc Sci 51, 3-14 (1996).

59. Lawton, M. P. The Philadelphia Geriatric Center Morale Scale: a revision. J Gerontol 30, 85-9 (1975).

60. Helmes, E., Csapo, K. G. & Short, J. A. Standardization and validation of the Multidimensional Observation Scale for Elderly Subjects (MOSES). J Gerontol 42, 395-405 (1987).

61. de Jonghe, J. F., Kat, M. G., Kalisvaart, C. J. & Boelaarts, L. [Neuropsychiatric inventory questionnaire (NPI-Q): A validity study of the Dutch form]. Tijdschr Gerontol Geriatr 34, 74-7 (2003).

62. Kaufer, D. I. et al. Validation of the NPI-Q, a brief clinical form of the Neuropsychiatric Inventory. J Neuropsychiatry Clin Neurosci 12, 233-9 (2000).

63. Cohen-Mansfield, J., Marx, M. S. & Rosenthal, A. S. A description of agitation in a nursing home. J Gerontol 44, M77-84 (1989).

64. De Jonghe, J. F. M. Factor structure and validity of the dutch version of the cohen-mansfield agitation inventory (CMAI-D). JAGS 44, 888-889 (1996).

65. Corder, E. H. et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921-3 (1993).

66. Poirier, J. et al. in Alzheimer Disease: Therapeutic Strategies (eds. Giacobini, E. & Becker, R.) 72-76 (Birkhauser, Boston, 1994).

67. Borroni, B. et al. ApoE genotype influences the biological effect of donepezil on APP metabolism in Alzheimer disease: evidence from a peripheral model. Eur Neuropsychopharmacol 12, 195-200 (2002).

68. Sjogren, M. et al. Tacrine and rate of progression in Alzheimer's disease--relation to ApoE allele genotype. J Neural Transm 108, 451-8 (2001).

69. Winblad, B. et al. A 1-year, randomized, placebo-controlled study of donepezil in patients with mild to moderate AD. Neurology 57, 489-95 (2001).

70. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. (American Psychiatric Press, Washington, DC, 1994).

Review section

Chapter 2

Functional plasticity of the circadian timing system in old age: light exposure

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Abstract

Circadian rhythms are present in virtually all physiological processes, and supposedly have adaptive value. In many healthy elderly, and even more so in demented elderly, rhythms become unstable and show a reduced amplitude. The present review describes how age-related degeneration in the core of the circadian timing system, the suprachiasmatic nucleus, and the consequent weak or altered output of the system, have negative implications for the health, sleep, mood, cognitive performance and overall well-being of healthy and demented elderly. Given these negative consequences, it is of importance to investigate which mechanisms underlie the degeneration of the circadian timing system in order to be able to develop rational treatment strategies. Although rhythms can be generated endogenously without any external stimulation, under normal conditions the circadian timing system is continuously exposed and responding to stimuli originating in the environment or in the body, including light, melatonin, physical activity, somatosensory stimulation and temperature. The present paper demonstrates how aging is associated with a diminishing input of environmental light to the circadian timing system. This condition of hypo-activation results not only in poor expression of rhythms but moreover accelerates neuronal shrinkage, paraphrased as 'use it or lose it'. Enforcing an increase in stimulation by means of bright light would be a rational way to supplement the hypo-activated circadian timing system. The last part of the review indeed confirms that bright light improves the expression of rhythms in core body temperature, melatonin and sleep-wakefulness in healthy and demented elderly. Thus, the circadian timing system indeed retains a high degree of plasticity at high age, and given the proper stimuli responds even in elderly suffering from Alzheimer's disease. It remains to be investigated whether the enhanced expression of rhythms also underlies the light-induced improvements in mood, behavioural disturbances and cognition, which can however be welcomed anyway as at least positive side effects of bright light treatment.

Introduction

The circadian timing system (CTS) has evolved in an environment where the rotation and orbit of the earth and moon resulted in continuing cyclic variations of light and temperature, now known as days, months and seasons. In mammals, the central coordinating pacemaker of the circadian timing system – the biological clock of the brain - is located in the hypothalamic suprachiasmatic nuclei (SCN), which are able to generate rhythms even in the complete absence of such cyclic environmental variations. This does not mean that environmental stimuli have lost all significance for the CTS. On the contrary, rhythms with reduced amplitudes and periods differing from 24 hours (free-running rhythms) may occur in the absence of cyclic environmental stimuli. In normal environmental conditions, the CTS continuously compares and integrates this input with its own oscillators and adapts if necessary. This is what allows us humans to adapt to self-induced phase shifts resulting from traveling across many time zones within a brief period of time. Experiments in which all time cues were eliminated have shown that the CTS not only adapts after such time zone crossings, but in fact every day in order to keep a mostly non-24 hour endogenous rhythm in line with the 24-hour environmental rhythms. Thus, the CTS is a system with a high degree of plasticity. The functional plasticity of the CTS at old age is defined for the present paper in its broadest sense [1], as the capacity of the aged CTS to respond to bright light in a way that supports its functionality during a time window not restricted to the duration of the application. For a discussion on plasticity of the CTS not limited to aging, the reader is referred to Amir et. al[2], who show many examples of long-last-

ing changes in e.g. arrhythmicity, phase and period after the application of light.

The present review focuses on the question to what extent the CTS and its plasticity are maintained at high age, especially in humans. During aging several aspects of the CTS change, and most of these changes are indicative of less than optimal adaptation. Other effective stimuli, like melatonin, temperature and physical activity are not discussed due to limitations on the length of this review, and are discussed in [3-6]. The first part of this review provides a brief background on the mechanisms involved in the endogenous generation of rhythms in the SCN, how these are affected by environmental light input, and to what extent the CTS is involved in the rhythms of core body temperature, hormones, mood, sleep-wakefulness and cognitive performance and age-related changes in these parameters. The second half of the review discusses (a) the functional anatomy and physiology of light input, (b) age-related input decrements, (c) the effects of experimental input deprivation and (d) the effect of light input suppletion on core body temperature, melatonin, mood and behaviour, sleep-wakefulness and cognitive performance. Collectively, the reviewed papers indicate that the CTS indeed retains a high degree of plasticity at old age, and given the proper stimuli responds even in elderly suffering from Alzheimer's disease.

The circadian timing system

The central pacemaker of all rhythms in the body is located in the hypothalamic SCN, representing the biological clock of the brain. The SCN consist of two small ($\pm$ 0.25 mm^2) nuclei located at the bottom of the anterior hypothalamus just above the optic chiasm and separated by the third ventricle. There has been considerable progress in our understanding of the in-put, output and oscillation mechanisms, of which a condensed and of necessity limited overview is given below.

Structure of the suprachiasmatic nuclei

Within the SCN several types of peptidergic neurons are found. As is the case in rat and monkey, the human SCN contains vasopressin (AVP) and vasoactive intestinal polypeptide (VIP)-producing neurons[7-9]. However, the largest cell population of the human SCN consists of neurotensin (NT) neurons, while only few of these cells are found in rat and monkey. Another difference is that the human SCN contains neuropeptide Y (NPY)-producing neurons[10]. These peptides are colocalized with gamma aminobutyric acid (GABA), which is present in all SCN neurons, but only in up to 38% of the terminals of projections from the SCN. GABA projections within the SCN could provide a cyclic inhibitory influence on other SCN neurons[11-15].

In the last decade, a clear progress has been made in understanding the molecular mechanism underlying the clock oscillation, which consists of gene-protein-gene feedback loops, where proteins can down regulate their own transcription and stimulate transcription of other clock proteins[16]. A discussion of the molecular mechanisms would take an extended review paper in itself, and is outside the scope of the present paper. Several excellent reviews on this topic have appeared[17-22].

Another topic that received considerable, renewed, interest in the last decade, is the concept of multiple oscillators both within the molecular clock mechanism[23], as well as within regions of the SCN[24] and even throughout the body[16]. Several observations suggest the existence of two oscillator groups in the SCN, one synchronized to sunrise at dawn (M), the other to sunset at dusk (E). For example, it is known already for some time that the evening onset and morning offset of two SCN-driven rhythms - pineal N-acetyltransferase activity and locomotor activity – can shift independently. Progress in the underlying mechanisms was recently made from in vitro SCN recordings with unusual slice orientations[24]. In the usual coronal slices only one peak in multi-unit neuronal activity (MUA) can be recorded, however in horizon-

tal slices two distinct peaks could be found. As compared to short days (LD 8:16), longer days (LD 14:10) elongated the duration of the morning peak, caused by an earlier onset, whereas no changes were found in the evening peak. Additional evidence was derived from the application of glutamate, the major transmitter conveying retinal information to SCN cells, which induces a transient increase in MUA. When applied early in the subjective night (CT 16), the evening but not morning peak delayed by 3 hours. When applied late in the subjective night (CT 0), the morning but not evening peak advanced by 2 hours.

Innervation of the suprachiasmatic nuclei

Of the inputs the SCN receives, the pathway of environmental light has been described the most comprehensively. The SCN receives information about the environmental light-dark cycle by a direct projection through the retinohypothalamic tract (RHT), for which glutamate is at present the most likely transmitter[25, 26]. A second, indirect input has been described in rats and monkeys. It runs from the retina to the intergeniculate leaflet, which projects to the hypothalamus with GABA and neuropeptide Y as neurotransmitters[26-28]. It is not certain whether this indirect pathway also exists in human.

Photic input reaches a subset of SCN cells largely restricted to the ventrolateral aspect in rats[29] and reaching more dorsally in hamsters[30]. This region of the SCN is referred to as the core, and is surrounded by the shell. The core projects densely to the shell, but little reciprocal innervation is found[29]. The topography of the human RHT projections has been described by Dai et. al.[25]. RHT fibers leave the optic chiasm and enter the hypothalamus medially and laterally at the anterior level of the SCN. The medial fibers enter the ventral part of the SCN and innervate the ventral SCN over its entire length, but the density decreases gradually from anterior to posterior. Labeled RHT fibers in the SCN make contact mainly with immunocytochemically positive NT or VIP neurons and only occasionally with vasopressin-positive neurons located in the ventral part of the SCN. Only few projections to the dorsal part of the SCN and the anteroventral part of the hypothalamus are present.

In addition to retinohypothalamic input, the SCN also receives inputs from other hypothalamic nuclei, the raphe, locus coeruleus, limbic forebrain and from the hormonal milieu[10, 15, 26, 31]. For example, the projection from the intergeniculate leaflet plays a crucial role in the effects of activity level on the circadian timing system[32-34]. Although lesions of these other incoming pathways affect circadian rhythms much less than a blockade of the transmission of light to the SCN does[35], they are thought to be of crucial importance in the entrainment of the circadian timing system by stimuli other than light, for example melatonin, temperature, somatosensory input and physical activity. A schematic overview of input to the SCN is given in figure 1.

Output of the suprachiasmatic nuclei

The number of projections leaving the hypothalamus is rather small. Animal studies suggest projections predominantly to other nuclei within the hypothalamus, as well as to the thalamus, basal forebrain and periaquaeductal grey[10, 36]. In rats, by far the densest SCN projections are to the subparaventricular zone, intermediate between the SCN and the paraventricular nucleus[36-39]. The area - referred to in literature as 'peri-SCN', 'subparaventricular zone' or 'area between the SCN and anteroventral part of the paraventricular nucleus'[28, 36, 40] - receives input from the SCN and has numerous projections. This area has therefore been proposed to amplify and distribute the circadian signals to effector systems. Human postmortem studies demonstrated projections comparable to those found in rodent and monkeys, to the subparaventricular zone, the periventricular nucleus, the paraventricular nucleus, the dorsomedial nucleus and the anteroventral hypothalamic area[40, 41].

Like the peptidergic and GABA-ergic projections within the SCN, projections to other hypothalamic nuclei are thought

to provide a cyclic inhibitory influence on neurons of the circadian effector systems and the SCN itself[11-15]. Moreover, projections to the intergeniculate leaflet and raphe, which both have afferents to the SCN[27], could provide a neuronal substrate for feedback loops. Projections to the anterior and posterior hypothalamus could be involved in arousal (sleep-wakefulness) and temperature regulating mechanisms which are co-localized in these parts of the hypothalamus[42]. The SCN projects to the pineal via the dorsomedial hypothalamic nucleus, the upper thoracic interomediolateral cell columns of the spinal cord and the superior cervical ganglia, resulting in the rhythmic secretion of melatonin[43, 44]. The presence of melatonin-receptors in the SCN[45] and the ability of melatonin to reset the electrical activity rhythm in *in vitro* SCN slices[46, 47] could provide a hormonal substrate for a feedback loop.

Like the differences in input to the core and shell subdivisions mentioned above, similar subdivisions are present for the output of the SCN to hypothalamic structures. The core projects predominantly to the lateral subparaventricular zone, the shell mainly to the medial subparaventricular zone and the dorsomedial nucleus[13]. In hamster, there is a compact subnucleus of calcium-binding protein calbindin-D28K (CaBBP) positive cells in the core. Its caudal position allows for selective lesions. Results of these studies suggest that the pacemaker in this subdivision is necessary and sufficient for the control of locomotor rhythmicity[30]. Subdivisions reflecting differential outputs have also been demonstrated in the human SCN[40, 41]. A schematic overview of the output of the SCN is given in figure 2.

Although the circadian oscillation of SCN neurons originates from an intracellular molecular loop which is not dependent on synaptic transmission, the output of the SCN as a whole is likely to depend on the synchronization of the rhythms of the individual cells, in which electrical activity may be of importance[48]

Age related changes in the circadian timing system

Age related changes in the suprachiasmatic nucleus

Animal studies indicate that the total number of neurons in the SCN does not decrease with age, and does not correlate with the circadian amplitude in running activity, eating and drinking[49]. There is however an age-related decrease in the number of cells that express AVP and VIP in the SCN[50, 51]. The aging human SCN is also characterized by decreased neuronal activity and expression of AVP[7, 52]. The number of VIP-expressing neurons declines after the age of 40 years only in males[53]. The circadian and circannual fluctuations observed in the number of SCN neurons expressing AVP disappears over the age of 50 years[54, 55]. A recent study demonstrated that not only the expression of AVP peptide, but also of AVP mRNA is affected in aging. AVP mRNA in the SCN of healthy controls shows a marked daytime (12:00-22:00 hour) increase, which is reduced in subjects over 80 years of age[56]. Other alterations in the SCN at high age include a decrease in glucose utilization[57], a loss in the concentration and rhythmic expression of alpha-1 adrenergic receptors[58].

Alzheimer's disease (AD) is associated with an even stronger and earlier decrease in markers of SCN neuronal activity. Pathologic changes consist mainly of shrinkage of cells, some neurofibrillary tangles and only rare diffuse plaques. Van de Nes et al.[59] found no amyloid-ß staining in the SCN of either AD patients or elderly controls, and only few neurofibrillary tangles. Hyperphosphorylated tau staining with Alz-50 indicated that cytoskeletal changes in perikarya and dystrophic neurites are present in the SCN of AD patients but not of elderly control subjects. As compared to the SCN of healthy elderly controls, the SCN of AD patients shows an even stronger decrease in the expression of AVP and NT, as well as an increase in glial fibrillary acidic protein (GFAP), indicating an

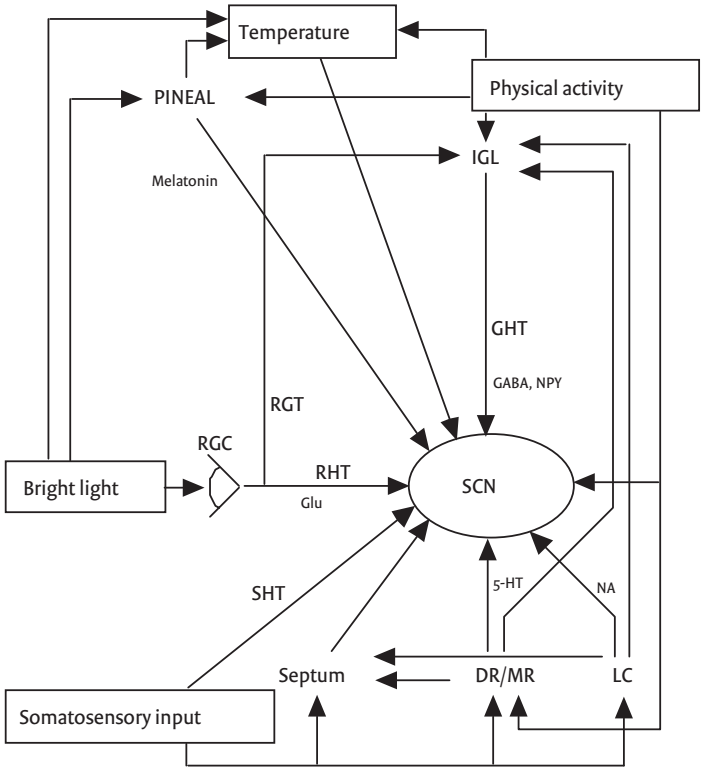

Figure 1.
Schematic and simplified overview of the inputs to the suprachiasmatic nucleus, and their interactions. Inputs are in outlined font, structures in bold, tracts in normal font and neurotransmitters and hormones in italics. Abbreviations: 5-HT=5-hydroxytryptamine (serotonin); DR=dorsal raphe nucleus; GABA=gamma aminobutyric acid; GHT=geniculohypothalamic tract; Glu=glutamate; IGL=intergeniculate leaflet; LC=locus coeruleus; MR=median raphe nucleus; NA=noradrenalin; NPY=neuropeptide Y; RGC=retinal ganglion cells; RGT=retinogeniculate tract; RHT=retinohypothalamic tract; SCN=suprachiasmatic nucleus; SHT=spinohypothalamic tract (from Van Someren et al. 1997).

increase in astrocytes[7, 60]. The expression of VIP is less affected in AD, only significantly so in a subgroup of presenile women[53]. The age-related reduction in daytime AVP mRNA is more pronounced in AD patients[56].

Age related changes in the output of the circadian timing system

The *amplitude* of circadian rhythms shows a strong decline with aging[61]. This finding has been confirmed in numerous studies at all levels of the CTS, ranging from rhythms in peptide expression in the SCN[54], to rhythms in hormone levels[62, 63] and even rhythms in cognitive performance[64]. The inter-individual variability in the amplitude increases, resulting in individual declines ranging from strong to absente.g.[65]. This variability may underlie the absence of an amplitude reduction found in some studies in very healthy elderly[66, 67]. Changes in the intrinsic free-running *period* have been reported, but remain equivocal[68-70]. On the other hand, many studies have confirmed an *advanced phase*[68-70]. For the body temperature rhythm it appears that especially the early morning rising phase of the curve is advanced in old age[64, 66]. The phase relation between the several rhythms also shows changes. For example, the onset of the activity period is advanced with respect to its timing on the morning rise in body temperature[64, 71], thus limiting the ability to maintain sleep in the morning. Moreover, the habitual sleep period is advanced with respect to the already advanced melatonin secretion period[72]. A decreased plasticity of the aged circadian system is evidenced by the reduced ability to re-*entrain* after a phase shift[73-75]. Possibly indicative of coping with this decreased ability to shift, most elderly

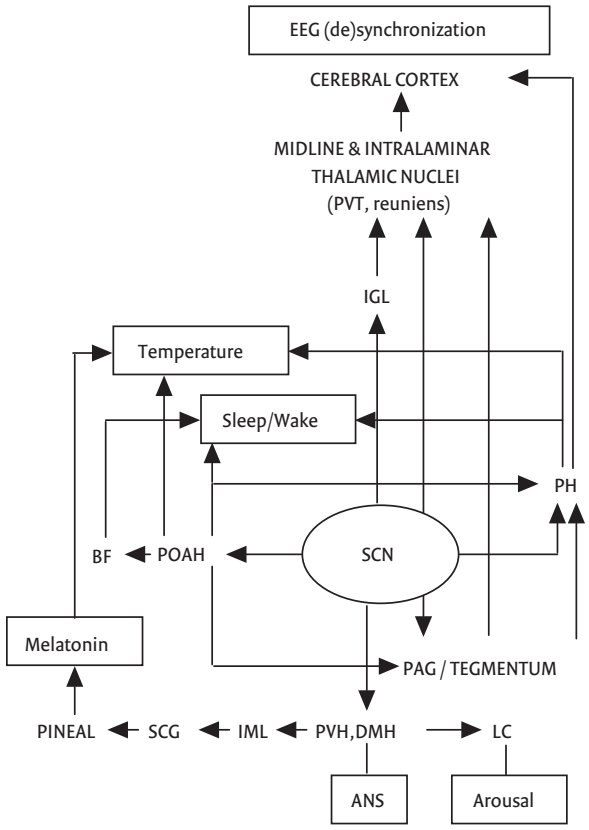

Figure 2.
Schematic and simplified overview of the output of the suprachiasmatic nucleus involved in the circadian modulation of the autonomic nervous system (ANS), arousal, hormones, temperature, the sleep-wake state and cortical (de)synchronization, all indicated in outlined font. Structure abbreviations: BF=basal forebrain; DMH=dorsomedial hypothalamic nucleus; LC=locus coeruleus; IML=upper thoracic interomediolateral cell columns of the spinal cord; PAG=periaquaductal grey; PH=posterior hypothalamic area; POAH=preoptic and anterior hypothalamic area; PVH=paraventricular hypothalamic nucleus; PVT=paraventricular thalamic nucleus; SCG=superior cervical ganglia; SCN=suprachiasmatic nucleus. For more details see text and [16, 283]

show an increased regularity in their daily patterning of sleep-wake times, meals and social interactions[76, 77]. On the other hand, a loss of regularity is the most clearly altered circadian variable in AD patients[78, 79]. Moreover, the loss of circadian rhythms in aging and dementia is associated with a relative increase in the expression of infradian and ultradian (quasi-)rhythmic components[78-80]. In comparison to mild, moderate and non-demented institutionalized elderly, severely demented patients sleep more both during the day and night and have a more blunted circadian activity rhythm[81].

There are numerous indications that the decrease in *vasopressin* expression in aging SCN neurons, as mentioned in the previous paragraph, is functionally implicated in the weakening of rhythms. AVP is one of the output signals of the clock and associated with the strength of the rhythm. In vitro, vasopressin-containing cells form a subpopulation of neurons with the most pronounced circadian rhythmicity in spontaneous firing rate[82]. AVP acts within the SCN in an excitatory way through V1 receptors, and enhances the circadian amplitude of the neuronal firing rate[83, 84]. Indeed, the pattern of SCN AVP secretion is correlated with both electrical and metabolic activity of SCN neurons[16]. AVP may not be essential for rhythm generation per se, since SCN grafts from genotypically AVP-deficient Brattleboro rats restore rhythms in SCN-lesioned arrhythmic Wistar rats[85]. On the other hand, it is conceivable that in the genotypically AVP-deficient Brattleboro rats, plasticity instigates the taking over of AVP-functions by other substances. Anyhow, the rhythms of VP-deficient Brattleboro rats are less robust

and show increased vulnerability: although a temperature rhythm is maintained under light-dark cycles, but in the absence of these Zeitgebers the temperature becomes irregular and non-rhythmic[86]. At the behavioural level, the loss in rhythm in aging voles is correlated with decreased AVP expression (M. Gerkema, unpublished data). In ground squirrels, the gradual recovery of circadian rhythms after waking up from hibernation is strongly correlated with the re-emergence of SCN neurons expressing AVP[48]. On the other hand, Jansen[87] reported increased AVP expression in the SCN of voles with *poor* overt rhythms. This apparently contradictory finding may however be interpreted as an accumulation due to a loss of transport or release. Unequivocal interpretation would require simultaneous assessment of mRNA and peptide levels.

Functional implications of weak or disturbed circadian rhythms

Circadian rhythms are thought to be of adaptive significance, allowing the organism to optimally tune the body functions to the environmental light-dark cycle. The evidence of a real evolutionary advantage of rhythmic modulation is however scarce, and in fact the adaptive significance of most rhythms remains to be demonstrated[88]. On the other hand there are numerous findings that indicate that a weak expression of circadian rhythms, as occurs in many elderly, is associated with poor functioning at several levels of which the present paragraph gives some examples relevant for aging, without being comprehensive.

Health and vital functions.

The longevity in hamsters is decreased with a non-invasive disruption of rhythmicity and is increased in older animals given fetal SCN implants that restore higher amplitude rhythms[89]. Resting heart rate shows a circadian rhythm under constant darkness and increases after light exposure[90]. The effect of light on heart rate is dependent both on the circadian phase of application and on its intensity. Cardiovascular problems may have more impact on the organism in the absence of a stable and strong circadian rhythm. Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic disease[91]. A flattened diurnal rhythm of heart rate in uncomplicated subjects with essential hypertension is a marker of increased risk for cardiovascular morbidity and mortality[92]. Long-term shift workers have a significantly increased risk for ischemic heart disease[93] and have less slow wave sleep and more subjective sleep complaints[94], and show temporal lobe atrophy and cognitive deficits[95-97]. Thermoregulation is also affected by a disturbed rhythm. The adaptation to cold stress is impaired in squirrel monkeys when their rhythms are not synchronized to a light-dark cycle[98].

Mood

Under controlled conditions, mood shows a significant circadian modulation[99,100]. There is ample evidence for a flattening of circadian amplitudes in depression[101, 102]. The severity of the depression is in fact negatively correlated to the amplitudes in hormone levels and core temperature, and remission is associated with a restoration of these amplitudes[103-105]. Recent post-mortem findings suggest that these correlations may indeed be traced back to the level of the SCN[106]. In SCNs obtained post-mortem from depressed subjects, the number of cells expressing the peptide AVP is increased, while the amount of AVP mRNA is decreased. These findings suggest that the synthesis, transport and release of AVP in the SCN are reduced, resulting in an impaired functional ability, which may contribute to the hyper activation of the hypothalamus-pituitary-adrenal (HPA) axis which is characteristic for depression. The corticotropin releasing hormone (CRH)-containing cells in the paraventricular nucleus that form the basis of this axis receive a strong inhibitory vasopressinergic projection from the SCN[107]. Thus, an impaired output of the SCN will

result in less inhibition and thus contribute to hyper activation of the HPA axis.

It should be noted that the relation between depression and circadian rhythm disturbances may also involve reverse causal processes. It is conceivable that the attenuated serotonergic system, which is characteristic for depression, has consequences for SCN functionality. The SCN receives direct serotonergic projections from the median raphe, as well as indirect projections from the dorsal raphe to the intergeniculate leaflet, which projects to the SCN. These inputs are involved in the presynaptic inhibitory modulation of the input the clock receives from the eyes through the RHT. In animals, a lesion of the median raphe induces changes in the circadian system consisting of an increased activity period at the cost of the resting period, and exaggerated phase shifts in response to bright light pulses[108]. If this model would hold for humans, it would be predicted that depressed patients with low serotonergic function are hyper responsive to light induced effects on the SCN.

The relation between depression and circadian disturbances is supported by our recent as yet unpublished findings that both depression ratings and saliva cortisol levels in demented elderly are correlated with the instability of the circadian activity rhythm.

Cognitive performance

The performance on cognitive tasks as well as evoked and average cortical activation patterns show circadian modulation[61, 109-111]. In addition, it is likely that age-related circadian rhythm disturbances contribute to the increase in memory problems with aging. It has been shown in rats and humans that a disruption of the circadian rhythm by means of enforcing either a phase shift or fragmentation induces retention problems[96, 112-121]. Circadian disturbances appear to disrupt most prominently the types of learning that are associated with exploratory behaviour and reward seeking[122]. A recent hippocampus-dependent place navigation study in rats suggests that initial acquisition robustly withstands circadian disturbances, and that problems arise specifically with later retrieval[114]. This finding argues for a disruption of consolidation processes, as has also been demonstrated in studies where sleep rather than the circadian rhythm was disturbed[123-126]. Direct and indirect projections from the SCN to the hippocampus[39, 127] may be involved. Involvement of the hippocampus is supported by a recent naturalistic study demonstrating that long-term exposure to jet-lag in female cabin crew is associated with temporal lobe atrophy[95]. Antoniadis and colleagues[122] demonstrated in hamsters that the age-related memory decline is not a general consequence of aging but occurs only in a subset of old hamsters that show a reduced amplitude in their activity rhythm. The link between circadian rhythm disturbances and memory deficits is furthermore supported by the fact that they are the most prominent and simultaneously developing symptoms in the senescence accelerated mouse line SAMP8[128]. Finally, in a patient with damage in the SCN region of the hypothalamus, circadian rhythm disturbances were accompanied by attenuated cognitive and behavioural functioning, once more suggesting that circadian organization may be essential for normal cognitive functioning[129].

Long term (> 5years) and frequent desynchronization of the endogenous and environmental rhythms as a result from crossing time zones in flight cabin crew is associated with a reduction in MRI-determined right temporal lobe volume[95, 96]. The reduction in size is strongly correlated with an elevation in cortisol levels. The affected subjects also showed longer reaction times and made more errors on a visuospatial task. Although not assessed in the studies of Cho et al.[95, 96], disturbed sleep may be involved in the increase in cortisol levels, and the latter in turn have been hypothesised to be involved in the development of hippocampal atrophy[130]. However, other findings argue against the latter mechanism: no evidence of hippocampal neuronal cell loss or other major morphological alterations could be demonstrated in post-mortem brains of patients that

had lived with elevated corticosteroid levels due to depression or corticosteroid treatment[131, 132]. It has been demonstrated that shift workers have less slow- wave sleep and more subjective sleep complaints[94], and that long-term (>5 years) sleep complaints are associated with increased cortisol levels[133, 134].

Also in both healthy and demented elderly the relation between a disturbed sleep-wake rhythm and clinically significant cognitive impairment has been demonstrated. In a sample of over 300 elderly, the occurrence of sleep disorders was found to be correlated with cognitive impairment, as indicated by the rating on the mini mental state evaluation (MMSE)[135]. In mentally and physically handicapped as well as geriatric inpatients, increased daytime and decreased night-time sleep was associated with more impairment in cognition and activities of daily living (ADL)[136, 137]. In demented elderly sleep disturbances predict a faster cognitive decline[138-140]. In fact, in demented elderly, the presence of nocturnal awakenings increases the odds ratio for institutionalization within a year by a factor 10[138].

Collectively, these data strongly suggest that weak or disturbed expression of circadian rhythms, as occurs in many elderly, may increase the occurrence and severity of adverse conditions for which elderly are already at risk, including mortality, cardiovascular problems, hypo- and hyperthermia, depression and cognitive decline. It would thus be of great value for the well-being of elderly to investigate the mechanisms involved in the deterioration of rhythms in order to develop rational measures aimed at restoration of rhythms. Our group has approached this investigation in a hypothesis based on the plasticity paradigm, paraphrased as 'use it or lose it'[141]. In brief, this hypothesis proposes that activation of neuronal systems promotes their resistance to age-related degenerative processes. The SCN is well suited for research on this hypothesis, since it is a structure in which plasticity seems highly preserved throughout the life span[142] and for which the possibilities to increase and decrease its activation are quite well described and feasible in human experimental research. These stimuli include light, melatonin, temperature, somatosensory input, and physical activity. The remainder of this review is focused on bright light and describes (1) how light affects neuronal activity in the SCN, (2) age-related changes in exposure to light, (3) consequences of a decreased light exposure and (4) consequences of increased exposure for core body temperature, melatonin, mood and behaviour, sleep-wakefulness and cognitive performance.

Light exposure and consequences for the circadian timing system

Functional anatomy

The primary Zeitgeber for the CTS is light falling on the eyes. Although one report suggested that extra-ocular light might be of relevance and induce phase-shifts[143], other studies could not confirm either melatonin suppression[144-146], or phase shifts[147, 148]. The retina contains ganglion cells with direct glutaminergic projections to the SCN through the RHT, and indirect projections via the thalamic intergeniculate leaflet. The classical rod and cone photoreceptor system is not necessary, although it is likely that, in intact animals, activation of ganglion cells by rods and cones does contribute. Recent studies are beginning to unravel the mechanism of the non-rod, non-cone photoreceptor system. A new opsin (melanopsin) which is expressed in ganglion and amacrine cell layers is likely to mediate the circadian photoreceptive pathway[149-155]. Other pigments (e.g. cryptochrome 1 and 2) may also be involved, but do not appear to be necessary. In humans, a similar non-rod, non-cone system is likely, since the SCN-mediated melatonin suppression by monochromatic light is most effective at 456 nm, followed in descending order by 472, 500, 520 and 548 nm, and this curve is not compatible with the absorption spectra of rods and cones[156]. Hankins and Lucas[157] estimated optimal sensitivity of this non-rod,

non-cone system in humans at 483 nm.

Photic input reaches the 'core' neurons of the SCN and induces release of glutamate from projecting retinal ganglion cells and consequently, membrane depolarization of SCN neurons[16]. In contrast to the adaptation characteristic of light input processed for visual information and projected to the occipital cortex, the 'photic' pathway to the SCN does not adapt, and remains activated with continuous illumination[158]. SCN neuronal firing rates increase over two to three orders of magnitude with increasing light intensity[158]. The actual increase induced, is dependent on the intensity as well as on the circadian phase of light application, with large responsiveness at night and low responsiveness during day[159, 160]. Whereas light induces an increase in firing rate throughout the circadian cycle, light induces gene expression only when applied during the subjective night (per-1 mRNA, c-fos and c-fos mRNA, jun B and jun B mRNA). The induction of phase-shifts is also limited to light applied during the subjective night. Increased mPer1 expression is a critical step for the induction of a phase shift[161].

Indirect projections to the SCN may also be of relevance. Although early tracing studies suggested that the retinohypothalamic tract projected exclusively to the SCN, recent studies using more sensitive tracers revealed projections to many hypothalamic areas including the preoptic and anterior hypothalamic areas, the lateral hypothalamic area, the subparaventricular zone, and the (ventro)lateral preoptic area[162-167]. In humans, lateral RHT projections reach the ventral part of the ventromedial SON and the area lateral to the SCN, but no projections were observed to other hypothalamic areas[25]. In animals many of the RHT-innervated hypothalamic nuclei in turn project to the SCN[162]. Retinal projections are not limited to hypothalamic areas, but have also been demonstrated to the basal forebrain cholinergic neurons[168], which project to the SCN[169], and are likely to be involved in the light-induced increase in acetylcholine concentration in the SCN[170]. Indeed, cholinergic agonists are capable of mimicking the effects of light[171]. Furthermore, there is a projection to the paraventricular thalamic midline nuclei which in turn project to the SCN[172]. Finally, several studies demonstrated retinal projections to the raphe nuclei, which in turn project to the SCN[173, 174]. Most studies are reviewed by Amir et al.[2].

Light may theoretically also be involved in *modulation of the output* from the SCN. The thalamic paraventricular nuclei receive input from both the retina and the SCN[38, 172]. The hypothalamic subparaventricular zone, one of the major relay projections from the SCN, also receives direct retinal projections[162, 163]. In man, there is a retinal projection to the hypothalamic paraventricular nucleus[175] which receives input from the SCN and is of major importance in the circadian modulation of the autonomic nervous system.

Effect of light on the expression of rhythms

Circadian effects of light. Circadian rhythms show a phase delay after light exposure early in the subjective night, and an advance after early morning light exposure. Phase advances are often preceded by transients unrelated to the ultimate advance, whereas delays often reach their ultimate value immediately[66, 176]. The ultimate shifts can be plotted against the times of application to generate a phase response curve (PRC).

Contrary to earlier beliefs, phase shifts may occur even with rather low light *intensities*. In a forced desynchrony study, domestic lighting (150-500 lux) induced phase shifts according to the light phase response curve[177]. The dose-response relationship between the light intensity and induced effect approximates a cube root function. This cube root gain step probably occurs early in the input-to-output stages of the CTS since it has been demonstrated in quite diverging output parameters, e.g. the phase shift by light[99] but also light-induced muscle sympathetic nerve activity[178].

The PRC shows a region of little response with application of light during the subjective day, and has traditionally been called the 'dead zone'. However, recent studies indicate

that the dead zone does not cover most of the day at all, and may in fact be very brief or absent. The presence or absence of bright light during the day has in fact consequences for several circadian rhythm parameters, as will be discussed later in more detail[179-181].

Acute effects of light. In addition to its effect on circadian rhythm parameters, light has a phase-dependent acute effect on many physiological functions that are used as indicators of the CTS. For example, light applied during the night elevates brain and core body temperature, whereas this effect is not present during the subjective day, both in Syrian hamsters[182] and humans[183]. However, in other studies, light applied in a thermoneutral to warm environment increased the vasodilatory gain and had the opposite effect of lowering brain temperature as measured at the tympanum[184, 185]. Nocturnal light suppresses melatonin, which has a heat loss promoting effect in humans. Thus a suppression of heat loss might be involved in the nocturnal increase in core temperature. Indeed, the light-induced increase in core temperature can be antagonized by suppletion of melatonin[186, 187]. On the other hand, independent of whether the core temperature increase is prevented by co-administration of melatonin, evening bright light still induces a phase delay in the circadian rhythms[186].

Age-related changes in light-induced activation of the SCN

Exposure to bright light. Elderly people, and, even more, AD patients, expose themselves to significantly less bright environmental light. Campbell et al.[188] and Savides et al.[189] investigated the time subjects were exposed to more than 2000 lux and reported average daily exposure times of 1.5 hour in healthy young adults, 1 hour in healthy elderly people and 0.5 hour in ambulatory, early stage AD patients. Maximum light exposure occurred between 12:00 and 13:00 hours in elderly people and AD patients and around 15:00 hours in young adults. Sanchez et al.[190] investigated the time subjects were exposed to more than 1000 lux and found daily averages of 1.7 hours in young adults and 0.6 hours in healthy elderly people. Shochat[191] reported that institutionalized elderly were exposed to a median light level of only 54 lux and were exposed to more than 1000 lux for only 10.5 min per day. Other studies have confirmed these findings, with only marginally different values[81, 180, 192]. In comparison to mild, moderate and non-demented institutionalized elderly, severely demented patients spend less time in bright light[81]. In addition, nightlights may result in a virtual absence of a day-night rhythm in light exposure in some nursing homes and homes for the elderly. In a study on 29 homes for the elderly, the average light intensity was 300 lux during daytime and 200 lux during the night[193].

Ocular transmission, retina and optic nerve. Of the little light elderly are exposed to, only a limited part is transmitted to the retina due to the age-related yellowing of the lens and the reduction in pupil diameter[194, 195]. Moreover, there is an age-related decrease in the densities of retinal ganglion cells[196] and of rods, but not cones[197]. In AD, the findings are equivocal. Many histological studies have reported a loss of retinal ganglion cells and optic nerve degeneration[198-200], although a few have not[196, 201]. In early stages AD patients show normal electroretinograms, the signal of which strongly depends on intact retinal ganglion cells[202]. Finally, elderly are at increased risk of ocular conditions that adversely affect light transmission, including cataract, glaucoma, macula degeneration and diabetic retinopathy[203, 204]. The risk of glaucoma is even more increased in elderly suffering from Alzheimer's or Parkinson's disease[205].

A recent study on the eyes of Aplysia throughout aging by Sloan et al.[206] has demonstrated that the age-related changes are indeed relevant for the circadian modulation of nerve impulses from the eye. Some older animals were characterized by clear lenses, intact retinas and intact rhythms. However, when the lenses were 'cloudy', cataract-like, the retina showed marked degeneration and the circadian pattern in neuronal output

showed an irregular and damped amplitude.

Central processing of photic information. The central processing of photic information by the CTS is attenuated at high age. Age-related changes observed post-mortem in the SCN have already been discussed above. It is very likely that the age-related loss of AVP in the SCN will result in an attenuated induction of SCN output changes in response to light stimulation.

In vivo studies have demonstrated that age affects the response to light exposure within the SCN. Light exposure during the subjective night induces expression of immediate early genes (IEG) in the SCN. In old animals photic stimulation induces is a decreased SCN response of the IEGs c-fos, and NGFI-A but not NGFI-B[207]. In aged and artificially aged gray mouse lemurs, more light is needed to reach the threshold for induction of c-fos expression[208]. Moreover, the aged animals need a much higher intensity of light to reach the saturation level of c-fos expression. This finding is contrary to what would be expected from the decreased habitual light exposure of elderly, since rats receiving a decreased habitual light exposure show in fact an enhanced SCN c-fos expression with light exposure[209].

In addition to the decrease in transmission of light input described above, and factors intrinsic to the SCN, the age-related decrease in acetylcholine (ACh) synthesis[210, 211] may also be involved in the attenuated the effect of light on the SCN. Light increases the ACh concentration in the SCN[170] possibly through retinal projections to the nucleus basalis magnocellularis[168], which in turn provide a strong cholinergic input to the SCN[169]. Both nicotinic and muscarinic receptors are present in the SCN, which decrease in number with increasing age[212]. Indeed, cholinergic agonists are capable of mimicking the effects of light[171]. Rats treated with a cholinergic neurotoxin show a phase advance and decreased amplitude, but no change in period[213], strikingly similar to the findings in normal aging. The phase-advance with a lack of ACh however is contrary to expectation, since ACh is only involved in phase advances[214], and a delay would thus be expected with a lack of ACh. In AD, which is characterized by a marked loss of cholinergic transmission, phase delays have indeed been reported (own unpublished observations and[215, 216]).

Phase response to bright light. In general, humans tolerate a phase delay, as occurs with travelling westwards across time zones, much better than a phase advance, as occurs with eastward travel. This preference is not altered in the elderly[217]. Light applied during the evening induces phase delays of similar magnitude in elderly as compared to younger subjects[218]. However, phase advances induced by light applied in the morning are less pronounced in the elderly. This is somewhat contrary to the expectation: many elderly show an advanced phase, and if this would be due to differential changes in the sensitivity for advances and delays in the biological clock, an attenuated response to delaying stimuli would have been predicted instead. Thus, the advanced phase of the circadian pacemaker in older people occurs in spite of a lower sensitivity to phase advances. It may be that early awakening and a preference to plan outdoor activities in the morning provide a daily phase advancing stimulus. Recent findings also suggest that a selective decrease in the sensitivity of the morning oscillator to phase delays may be involved in the phase advance and early awakening often found in elderly. Whereas evening bright light equally delays the melatonin *onset* by over one hour in both young and aged humans, the delay in melatonin *offset* is attenuated in elderly[219].

Effects of deprivation of a light-dark cycle

The suprachiasmatic nuclei. Whereas the importance of light-induced retinal input to the mammalian SCN was first described only as recent as 1972[220], SCN atrophy following eye removal was already documented in the forties, indicating a dependence on light input for survival of the structure[221, 222]. Less rigorous changes in light input also affect the SCN. As compared to daily light exposure for a long duration (16 h light/day), a short (10 h light/day) photoperiod suppresses AVP

mRNA and VIP mRNA in Syrian hamsters[223].

Expression of rhythms. A *complete lack* of light-induced activation of the CTS is associated with poor expression of rhythms. In rabbits, blinding by optic nerve sectioning results in a shortening of the free-running period[224]. In hamsters, lesioning the RHTt results in free-running rhythms with apparently increased fragmentation[225]. In humans, the absence of an environmental light-dark cycle (plus any other cyclic Zeitgeber) induces rhythms to express a near 24 hour 'free-running' period. The period is usually slightly longer than 24 hours. This implies that synchronization to the environmental light-dark cycle under naturalistic conditions is more dependent on daily phase advances than on phase delays. A study on forced continuous bed rest and elimination of daylight for more than two weeks in middle-aged subjects showed a delayed phase and reduced amplitude of the circadian rhythm in rectal temperature, difficulties falling asleep, and poor subjective sleep quality[226]. Totally blind subjects suffer more from disturbed sleep than partially blind subjects[227]. In completely blind subjects, free-running rhythms frequently occur. In blind subjects in their thirties and forties who were followed for over a decade, the period length of their free-running rhythm showed a slight age-related increase of about 6 minutes over one decade, contrary to the believe of a shortening of the endogenous rhythm with aging[228].

Several studies indicate that not only complete deprivation of light, but also *partial deprivation* is detrimental to the expression of circadian rhythms especially at high age. A health survey in almost 15.000 inhabitants of the municipality of Tromso which is located north of the Arctic Circle where the winters are extremely dark, midwinter insomnia is present in 17.6% of the women and 9.0% of the men[229]. Like in the experimental deprivation study of Monk[226], difficulties falling asleep were the most common type of insomnia. As compared to young adults, the sleep disturbances of middle aged subjects were more sensitive to decreased light exposure: insomnia occurred more frequently in the autumn, winter and spring, but *less* frequently in the summer. In institutionalized elderly, a lack of light exposure predicted more nocturnal awakenings and an earlier activity acrophase[191]. Light and activity rhythms were related in a lagged way, with maximum illumination preceding peak activity. Thus the level of daytime light exposure affects both night-time sleep consolidation and the timing of peak activity level. Especially at higher age, retinitis pigmentosa (RP), but not cataract, is associated with subjectively disturbed nocturnal sleep and daytime alertness, suggesting involvement of the degeneration of photoreceptive cells in the retina in an attenuated sleep-wake rhythm[230, 231]. In fact 76% of all evaluated RP patients and even 95% of the RP-patients older than 50 years of age could be classified as suffering from insomnia. These figures are much higher than the numbers found for blind people with partial light perception (±50%)[227], supporting the importance of retinal function rather than e.g. intact acuity.

In addition to the detrimental effect of partial and complete deprivation, it appears to be of crucial importance that light exposure occurs alternating in a circadian pattern. In rats, prolonged continuous exposure to light may initially cause uncoupling of activity and temperature rhythms, followed by a complete loss of circadian cycles and the emergence of ultradian cycles[232]. Free-running circadian rhythms are restored when the animals are placed in constant darkness. Similarly, under constant bright light and high ambient temperature (34˚C) the domestic fowl loses its circadian rhythm in body temperature[233]. It should furthermore be noted that the waveform of the light intensity cycle may strongly affect behavioural rhythms[234].

In summary, a lack of alternating exposure to bright light and darkness is associated with poor expression of circadian rhythms. In humans, the rhythm in sleep andwakefulness is strongly affected.

Elderly appear to be more sensitive to the detrimental effects of poor light-induced stimulation of the SCN than young subjects are. That these findings are of more than academic interest is evidenced by a recent study of Jean-Louis et al.[235] who demonstrated that the subjective quality of life in healthy middle aged subjects is positively correlated with their average level of exposure to illumination.

Effect of additional light input on circadian rhythms in the elderly

The finding that increased light exposure restores the age-related decrease in AVP expressing neurons in the SCN of old rats[236] suggests that the poor expression of many rhythms in elderly may be enhanced by application of bright light. The present paragraph reviews the effects of bright light treatment in elderly, as expressed in their core body temperature, melatonin, sleep-wake rhythm, mood, behavioural disturbances and cognition. The effects sorted indicate that plasticity is preserved in the CTS even at high age.

Core body temperature

In elderly women, morning bright light did non advance their core body temperature rhythm[237]. On the other hand, Campbell and Dawson[238] report a 1.5-hour delay in the body temperature nadir after evening bright light in elderly insomniacs. It is likely that a differential loss of the sensitivity to the phase-advancing effects of light are involved in these contrasting findings[218]. In addition to an effect on the phase of the core body temperature rhythm, bright light affects its amplitude. Bright day light has the delayed effect of a stronger decline of core temperature during the first half period of night sleep[180, 239, 240].

Melatonin

Old mice kept at 100-200 lux instead of 10 lux show improved stability and (re-) synchronization of their metabolic and endocrine rhythms[241]. Both in healthy subjects[179, 239, 242] and in poor sleeping elderly[180], bright light treatment during the day enhances melatonin release during the subsequent night. After daytime light, the onset of melatonin release at the beginning of the night advances, whereas the offset at the end of the night does not[179]. Given the heat loss-promoting properties of melatonin, this finding may underlie the stronger nocturnal decline of core temperature following bright light exposure during the day.

Whereas the nocturnal melatonin level is decreased with aging and demented elderly, the daytime level is increased[243-250]. Two hours of morning bright light (3000 lux) decreased daytime melatonin levels in psychiatric non-demented elderly but did not affect daytime melatonin in AD patients[251].

Sleep-wake and rest-activity rhythms

In old *rats*, increased light exposure restores the circadian amplitude of sleep-wakefulness to the level of young rats[252].

In *healthy elderly*, morning bright light improved self-evaluated sleep maintenance and quality[237] and reduced alertness at bedtime[253].

Campbell and Dawson[254] report more slow wave sleep, less stage transitions and less wake time after sleep onset in *elderly insomniacs* after evening exposure to bright light of 4000 to 5000 lux. Poor sleeping elderly also improved with midday light[180]. With respect to insomnia it is of interest that in young women evening bright light attenuated the low frequency but not high frequency component of the heart rate variability during subsequent sleep[255]. This finding suggests that daytime light can induce a reduction in nocturnal sympathetic activity, which is of clinical interest because insomniacs show an increased low frequency component and decreased high frequency component in their heart rate variability during sleep, which is indicative of hyperactivity of the sympathetic nervous system activity[256].

Van Someren et al.[78] were the first to demonstrate that disturbances in the rest-activity rhythm were more severe in *demented elderly* that were exposed to less bright light. A Japanese research group first reported a

reduction in sleep disturbances in about 50% of a group of 24 demented patients after bright light treatment[257]. The findings were confirmed in many subsequent studies[181, 258-266]. Once more, these findings indicate that even in demented elderly subjects functional plasticity of the CTS is preserved. An example is shown in figure 3.

Patients with more severe behavioural and sleep disturbances showed the greatest improvement[238, 264]. Two studies reported no effect of bright light[267, 268]: in one of the studies this was due to less than optimal analysis methods[79]. It should be noted that most studies were of short duration, with a maximum of three months[258]. To what extent the application of bright light remains valuable over a period of years is the topic of one of our present studies.

The time of day of the treatment does not appear to be critical. Positive effects on the sleep-wake rhythm were reported after exposure in the morning as well as in the evening, but also after exposure during lunch-time[255] or during the whole day[181].

Equivocal results have been reported considering the question whether poor ocular light transmission may attenuate light effects. Van Someren et al.[181] found no improvement of the rest-activity rhythm in demented patients with severe untreated cataract or loss of one eye, whereas those with intact eyes responded favourably. On the contrary Fukuda et al.[269] found improved sleep, indicated by an increase in stage 2 and a decrease in wakefulness as assessed by polysomnography, in patients suffering from mild to severe cataract. A comparison group was not present in the later study, and the number of afflicted subjects was small in both studies.

Mood and behavioural disturbances

The application of bright light to alleviate depression in seasonal affective disorder[270], is by now well accepted. However, also in non-seasonal depression[271] and in healthy people[272] light improves mood and vitality and alleviates distress. Morning bright light improved self-evaluations for alertness, mood, motivation, happiness, refreshment, concentration and appetite in elderly women[237]. Even a relatively minor improvement of the average illumination level at home improved mood in healthy elderly[273].

Mood improved with light therapy in elderly nursing home residents[274]. Bright light improves depression scores in institutionalized elderly, especially in the most depressed, which are also the ones that have been institutionalized for the longest time[275]. The application of bright light in depressed institutionalized elderly is of clinical relevance since depression onset increased the likelihood of death within a year by nearly 60%[275]. In one randomized controlled trial in demented elderly, one hour of morning bright light did not improve disturbances in behaviour and mood as assessed with the Behave-AD and Cornell scales respectively[260]. It should be noted that it is likely that not only circadian but also direct activating effects are involved.

Not only mood, but also agitated behaviour improves with bright light in about half of the treated demented elderly[257, 259, 262, 263, 276, 277]. The response in mildly demented subjects is better than in more severely demented subjects[276]. Evening bright light also improved the afternoon agitation known as 'sundowning' in AD patients[254, 264].

Cognition

Prolonged evening bright light treatment in elderly subjects suffering sleep maintenance insomnia enhanced their performance on a cognitive task battery[278]. The improvements were significantly related to sleep improvements, but not to the induced phase delay of the body temperature rhythm.

Recent studies indicate that bright light therapy in demented elderly improves the daytime cognitive performance[276, 279, 280], especially in mild dementia and less so in moderate to severe dementia[276]. It remains to be investigated to what extent improvements in cognition are accounted for by improvements in rhythms. Direct activating effects may be involved as well, since bright light increases alertness in humans[237, 281].

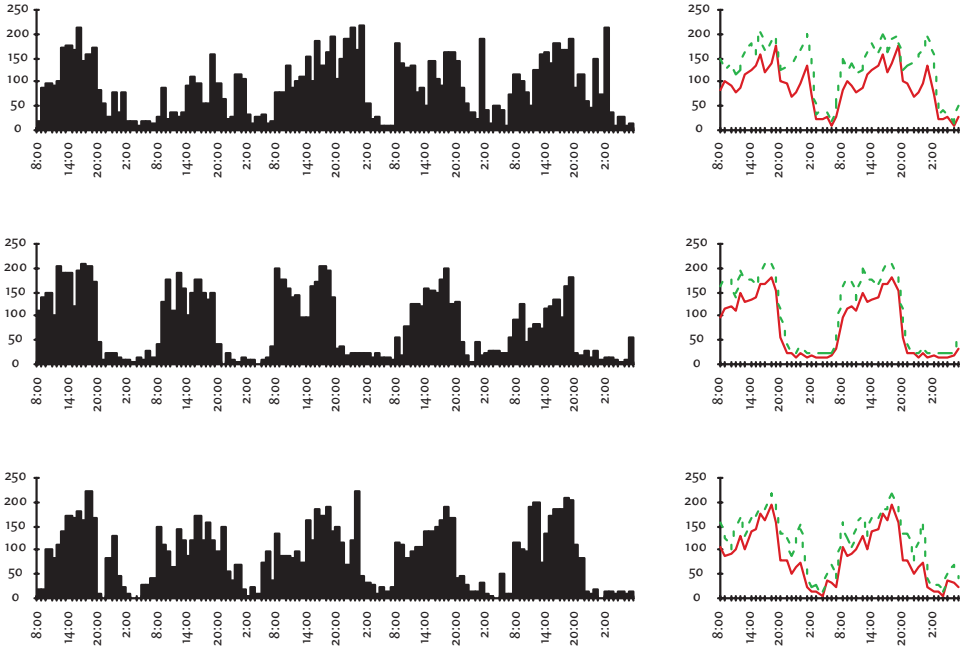

Figure 3.
Raw activity data (left panels) of a patient with Alzheimer's disease assessed three times for five days: before (upper left panel), during (middle left panel) and after (lower left panel) light treatment. The right panels show double plots of the average 24-hour activity level (solid line) and one standard deviation above this level (dashed line). Note the decreased variability and the smoother average during light treatmentv[181].

Conclusion

The present review indicates that the compromised strength of circadian rhythm expression in elderly has negative consequences for health. However, the CTS still shows high responsiveness and plasticity to supplementation of stimuli normally involved in synchronizing the endogenous and exogenous rhythms. The functional plasticity is evidenced by the capacity of the aged CTS to respond to bright light in a way that supports its functionality. Collectively, the reviewed papers indicate that the CTS indeed retains a high degree of plasticity at high age, and given the proper stimuli responds even in elderly suffering from AD. However, biochemical indications of this plasticity are sparse, and deserve more attention. In one study, increased light exposure restored the age-related decrease in AVP expressing neurons in the SCN of old rats[236]. In a simulation study, entrainment to a light-dark cycle stabilized the phase and enhanced the robustness of the molecular oscillatory mechanism[282]. Clearly, more studies are needed in order to determine the time course of preservation of the induced improvements. An important finding is that for many outcome parameters, the time of day of light application is of marginal, if any, importance in the effects sorted. This argues against the long-held belief that light-induced changes in rhythms result mainly from phase-shifts. Indeed, sleep improvements may occur without shifts in the rhythms[237]. Rather than a phase correction, an increase in the amplitude, i.e. strength of output of the circadian oscillator, appears to be involved. Of note with respect to this are the findings that daytime light has delayed effects during nocturnal sleep, including increased plasma melatonin levels and decreased core temperature. These findings indicate that light

not only has acute effects, but also strongly affects the circadian oscillating system. It remains to be demonstrated whether light keeps it's supportive effect when applied for years instead of weeks, as in all studies reported up till now. At least in short term studies, there is convincing evidence from the cellular to the behavioural level that proper stimuli like light exposure can trigger even cells and systems that are affected by aging and AD to show functional recovery.

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References

1. Lebeer, J. How much brain does a mind need? Scientific, clinical, and educational implications of ecological plasticity. Dev Med Child Neurol 40, 352-7 (1998).

2. Amir, S., Beaule, C., Arvanitogiannis, A. & Stewart, J. Modes of plasticity within the mammalian circadian system. Prog Brain Res 138, 191-203 (2002).

3. Van Someren, E. J. More than a marker: interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. Chronobiol Int 17, 313-54 (2000).

4. Van Someren, E. J., Lijzenga, C., Mirmiran, M. & Swaab, D. F. Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. J Biol Rhythms 12, 146-56 (1997).

5. Van Someren, E. J., Scherder, E. J. & Swaab, D. F. Transcutaneous electrical nerve stimulation (TENS) improves circadian rhythm disturbances in Alzheimer disease. Alzheimer Dis Assoc Disord 12, 114-8 (1998).

6. Van Someren, E. J. W., Scherder, E. J. A. & Swaab, D. F. in Alzheimer's Disease and Related Disorders (eds. Iqbal, K., Swaab, D. F., Winblad, B. & Wisniewski, H. M.) 771-779 (John Wiley & Sons Ltd, 1999).

7. Swaab, D. F., Fliers, E. & Partiman, T. S. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res 342, 37-44 (1985).

8. Swaab, D. F., Hofman, M. A. & Honnebier, M. B. Development of vasopressin neurons in the human suprachiasmatic nucleus in relation to birth. Brain Res Dev Brain Res 52, 289-93 (1990).

9. Swaab, D. F., Zhou, J. N., Ehlhart, T. & Hofman, M. A. Development of vasoactive intestinal polypeptide neurons in the human suprachiasmatic nucleus in relation to birth and sex. Brain Res Dev Brain Res 79, 249-59 (1994).

10. Moore, R. Y. The fourth C.U. Ariens Kappers lecture. The organization of the human circadian timing system. Prog Brain Res 93, 99-115 (1992).

11. Aronson, B. D. et al. Circadian rhythms. Brain Res Brain Res Rev 18, 315-33 (1993).

12. Buijs, R. M., Hou, Y. X., Shinn, S. & Renaud, L. P. Ultrastructural evidence for intra- and extranuclear projections of GABAergic neurons of the suprachiasmatic nucleus. J Comp Neurol 340, 381-91 (1994).

13. Buijs, R. M., Wortel, J. & Hou, Y. X. Colocalization of gamma-aminobutyric acid with vasopressin, vasoactive intestinal peptide, and somatostatin in the rat suprachiasmatic nucleus. J Comp Neurol 358, 343-52 (1995).

14. Gao, B. & Moore, R. Y. Glutamic acid decarboxylase message isoforms in human suprachiasmatic nucleus. J Biol Rhythms 11, 172-9 (1996).

15. Moore, R. Y. in Ciba Foundation Symposium 183. Circadian clocks and their adjustment (eds. Chadwick, D. J. & Ackrill, K.) 88-106 (John Wiley and Sons, 1995).

16. Buijs, R. M. & Kalsbeek, A. Hypothalamic integration of central and peripheral clocks. Nat Rev Neurosci 2, 521-6. (2001).

17. Albrecht, U. Invited review: regulation of mammalian circadian clock genes. J Appl Physiol 92, 1348-55 (2002).

18. Brown, S. A. & Schibler, U. The ins and outs of circadian timekeeping. Curr Opin Genet Dev 9, 588-94 (1999).

19. Cermakian, N. & Sassone-Corsi, P. Multilevel regulation of the circadian clock. Nat Rev Mol Cell Biol 1, 59-67 (2000).

20. Harmer, S. L., Panda, S. & Kay, S. A. Molecular bases of circadian rhythms. Annu Rev Cell Dev Biol 17, 215-53 (2001).

21. Reppert, S. M. & Weaver, D. R. Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 63, 647-76 (2001).

22. van Esseveldt, K. E., Lehman, M. N. & Boer, G. J. The suprachiasmatic nucleus and the circadian time-keeping system revisited. Brain Res Brain Res Rev 33, 34-77 (2000).

23. Daan, S. et al. Assembling a clock for all seasons: are there M and E oscillators in the genes? J Biol Rhythms 16, 105-16 (2001).

24. Jagota, A., de la Iglesia, H. O. & Schwartz, W. J. Morning and evening circadian oscillations in the suprachiasmatic nucleus in vitro. Nat Neurosci 3, 372-6 (2000).

25. Dai, J., Van der Vliet, J., Swaab, D. F. & Buijs, R. M. Human retinohypothalamic tract as revealed by in vitro postmortem tracing. J Comp Neurol 397, 357-70 (1998).

26. Moore, R. Y. Entrainment pathways and the functional organization of the circadian system. Prog Brain Res 111, 103-19 (1996).

27. Meijer, J. H. & Rietveld, W. J. Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. Physiol Rev 69, 671-707 (1989).

28. Rietveld, W. J. Neurotransmitters and the pharmacology of the suprachiasmatic nuclei. Pharmacol Ther 56, 119-30 (1992).

29. Leak, R. K., Card, J. P. & Moore, R. Y. Suprachiasmatic pacemaker organization analyzed by viral transynaptic transport. Brain Res 819, 23-32 (1999).

30. LeSauter, J. & Silver, R. Localization of a suprachiasmatic nucleus subregion regulating locomotor rhythmicity. J Neurosci 19, 5574-85 (1999).

31. Moore, R. Y. in 19th International Summer School of Brain Research (eds. Buijs, R. M., Kalsbeek, A., Mirmiran, M., Pennartz, C. M. & Romijn, H. J.) 55-57 (Netherlands Institute for Brain Research, Amsterdam, 1995).

32. Janik, D. & Mrosovsky, N. Intergeniculate leaflet lesions and behaviorally-induced shifts of circadian rhythms. Brain Res 651, 174-82. (1994).

33. Johnson, R. F., Smale, L., Moore, R. Y. & Morin, L. P. Lateral geniculate lesions block circadian phase-shift responses to a benzodiazepine. Proc Natl Acad Sci U S A 85, 5301-4 (1988).

34. Wickland, C. & Turek, F. W. Lesions of the thalamic intergeniculate leaflet block activity-induced phase shifts in the circadian activity rhythm of the golden hamster. Brain Res 660, 293-300 (1994).

35. Rietveld, W. J. & Groos, G. A. The role of suprachiasmatic nucleus afferents in the central regulation of circadian rhythms. Prog Clin Biol Res 59C, 205-11 (1981).

36. Watts, A. G. in Suprachiasmatic Nucleus. The Mind's Clock (eds. Klein, D. C., Moore, R. Y. & Reppert, S. M.) 77-106 (Oxford University Press, New York, 1991).

37. Lu, J. et al. Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. J Neurosci 21, 4864-74 (2001).

38. Watts, A. G. & Swanson, L. W. Efferent projections of the suprachiasmatic nucleus: II. Studies using retrograde transport of fluorescent dyes and simultaneous peptide immunohistochemistry in the rat. J Comp Neurol 258, 230-52 (1987).

39. Watts, A. G., Swanson, L. W. & Sanchez-Watts, G. Efferent projections of the suprachiasmatic nucleus: I. Studies using anterograde transport of Phaseolus vulgaris leucoagglutinin in the rat. J Comp Neurol 258, 204-29 (1987).

40. Dai, J., Swaab, D. F., Van der Vliet, J. & Buijs, R. M. Postmortem tracing reveals the organization of hypothalamic projections of the suprachiasmatic nucleus in the human brain. J Comp Neurol 400, 87-102 (1998).

41. Dai, J., Swaab, D. F. & Buijs, R. M. Distribution of vasopressin and vasoactive intestinal polypeptide (VIP) fibers in the human hypothalamus with special emphasis on suprachiasmatic nucleus efferent projections. J Comp Neurol 383, 397-414 (1997).

42. Miller, J. D. On the nature of the circadian clock in mammals. Am J Physiol 264, R821-32 (1993).

43. Cagnacci, A., Elliott, J. A. & Yen, S. S. C. Melatonin: A Major Regulator of the Circadian Rhythm fo Core Temperature in Humans. Journal of Clinical Endocrinolgy and Metabolism 75, 447-452 (1992).

44. Kalsbeek, A. et al. GABA Receptors in the Region of the Dorsomedial Hypothalamus of Rats Are Implicated in the Control of Melatonin and Corticosterone Release. Neuroendocrinology 63, 69-78 (1996).

45. Weaver, D. R., Stehle, J. H., Stopa, E. G. & Reppert, S. M. Melatonin receptors in human hypothalamus and pituitary: implications for circadian and reproductive responses to melatonin. J Clin Endocrinol Metab 76, 295-301. (1993).

46. McArthur, A. J., Gillette, M. U. & Prosser, R. A. Melatonin directly resets the rat suprachiasmatic circadian clock in vitro. Brain Res 565, 158-61 (1991).

47. Starkey, S. J., Walker, M. P., Beresford, I. J. & Hagan, R. M. Modulation of the rat suprachiasmatic circadian clock by melatonin in vitro. Neuroreport 6, 1947-51 (1995).

48. Hut, R. A., Van der Zee, E. A., Jansen, K., Gerkema, M. P. & Daan, S. Gradual reappearance of post-hibernation circadian rhythmicity correlates with numbers of vasopressin-containing neurons in the suprachiasmatic nuclei of European ground squirrels. J Comp Physiol [B] 172, 59-70 (2002).

49. Peng, M. T., Jiang, M. J. & Hsu, H. K. Changes in running-wheel activity, eating and drinking and their day/night distributions throughout the life span of the rat. J Gerontol 35, 339-47 (1980).

50. Chee, C. A., Roozendaal, B., Swaab, D. F., Goudsmit, E. & Mirmiran, M. Vasoactive intestinal polypeptide neuron changes in the senile rat suprachiasmatic nucleus. Neurobiol Aging 9, 307-12 (1988).

51. Roozendaal, B., van Gool, W. A., Swaab, D. F., Hoogendijk, J. E. & Mirmiran, M. Changes in vasopressin cells of the rat suprachiasmatic nucleus with aging. Brain Res 409, 259-64 (1987).

52. Swaab, D. F. et al. Tau and ubiquitin in the human hypothalamus in aging and Alzheimer's disease. Brain Res 590, 239-49 (1992).

53. Zhou, J. N., Hofman, M. A. & Swaab, D. F. VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. Neurobiol Aging 16, 571-6 (1995).

54. Hofman, M. A. & Swaab, D. F. Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res 651, 134-42 (1994).

55. Hofman, M. A. & Swaab, D. F. Influence of aging on the seasonal rhythm of the vasopressin-expressing neurons in the human suprachiasmatic nucleus. Neurobiol Aging 16, 965-71 (1995).

56. Liu, R. Y. et al. Decreased vasopressin gene expression in the biological clock of Alzheimer disease patients with and without depression. J Neuropathol Exp Neurol 59, 314-22 (2000).

57. Wise, P. M., Cohen, I. R., Weiland, N. G. & London, E. D. Aging alters the circadian rhythm of glucose utilization in the suprachiasmatic nucleus. Proc Natl Acad Sci U S A 85, 5305-9. (1988).

58. Weiland, N. G. & Wise, P. M. Aging progressively decreases the densities and alters the diurnal rhythms of alpha 1-adrenergic receptors in selected hypothalamic regions. Endocrinology 126, 2392-7 (1990).

59. van de Nes, J. A., Kamphorst, W., Ravid, R. & Swaab, D. F. Comparison of beta-protein/A4 deposits and Alz-50-stained cytoskeletal changes in the hypothalamus and adjoining areas of Alzheimer's disease patients: amorphic plaques and cytoskeletal changes occur independently. Acta Neuropathol (Berl) 96, 129-38 (1998).

60. Stopa, E. G. et al. Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. J Neuropathol Exp Neurol 58, 29-39 (1999).

61. van Someren, E. J., Mirmiran, M. & Swaab, D. F. Non-pharmacological treatment of sleep and

wake disturbances in aging and Alzheimer's disease: chronobiological perspectives. Behav Brain Res 57, 235-53 (1993).

62. Copinschi, G. & Van Cauter, E. Effects of ageing on modulation of hormonal secretions by sleep and circadian rhythmicity. Horm Res 43, 20-4 (1995).

63. van Coevorden, A. et al. Neuroendocrine rhythms and sleep in aging men. Am J Physiol 260, E651-61 (1991).

64. Duffy, J. F., Dijk, D. J., Klerman, E. B. & Czeisler, C. A. Later endogenous circadian temperature nadir relative to an earlier wake time in older people. Am J Physiol 275, R1478-87 (1998).

65. Li, H. & Satinoff, E. Changes in circadian rhythms of body temperature and sleep in old rats. Am J Physiol 269, R208-14 (1995).

66. Monk, T. H. & Kupfer, D. J. Circadian rhythms in healthy aging--effects downstream from the pacemaker. Chronobiol Int 17, 355-68 (2000).

67. Zeitzer, J. M. et al. Do plasma melatonin concentrations decline with age? Am J Med 107, 432-6 (1999).

68. Czeisler, C. A. et al. Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 284, 2177-81 (1999).

69. Mailloux, A., Benstaali, C., Bogdan, A., Auzeby, A. & Touitou, Y. Body temperature and locomotor activity as marker rhythms of aging of the circadian system in rodents. Exp Gerontol 34, 733-40 (1999).

70. Weitzman, E. D., Moline, M. L., Czeisler, C. A. & Zimmerman, J. C. Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. Neurobiol Aging 3, 299-309 (1982).

71. Dijk, D. J., Duffy, J. F. & Czeisler, C. A. Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep. Chronobiol Int 17, 285-311 (2000).

72. Duffy, J. F. et al. Peak of circadian melatonin rhythm occurs later within the sleep of older subjects. Am J Physiol Endocrinol Metab 282, E297-303 (2002).

73. Quay, W. B. Pineal homeostatic regulation of shifts in the circadian activity rhythm during maturation and aging. Trans N Y Acad Sci 34, 239-54 (1972).

74. Rosenberg, R. S., Winter, J. B. & Rechtschaffen, A. Effect of light phase reversal on sleep rhythms of young an old rats. Sleep Research 9, 105 (1990).

75. Zee, P. C., Rosenberg, R. S. & Turek, F. W. Effects of aging on entrainment and rate of resynchronization of circadian locomotor activity. Am J Physiol 263, R1099-103 (1992).

76. Minors, D. S. & Waterhouse, J. The role of naps in alleviating sleepiness during an irregular sleep-wake schedule. Ergonomics 30, 1261-1273 (1987).

77. Monk, T. H., Reynolds, C. F., 3rd, Machen, M. A. & Kupfer, D. J. Daily social rhythms in the elderly and their relation to objectively recorded sleep. Sleep 15, 322-9 (1992).

78. van Someren, E. J. et al. Circadian rest-activity rhythm disturbances in Alzheimer's disease. Biol Psychiatry 40, 259-70. (1996).

79. Van Someren, E. J. et al. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int 16, 505-18 (1999).

80. Gubin, D. G. & Gubin, G. D. A circadian to extra-circadian variance transposition of human body temperature with advancing age. Chronobiol Int 16, S41 (1999).

81. Ancoli-Israel, S. et al. Variations in circadian rhythms of activity, sleep, and light exposure related to dementia in nursing-home patients. Sleep 20, 18-23 (1997).

82. Schaap, J. et al. Neurons of the rat suprachiasmatic nucleus show a circadian rhythm in membrane properties that is lost during prolonged whole-cell recording. Brain Res 815, 154-66 (1999).

83. Ingram, C. D. et al. Vasopressin neurotransmission and the control of circadian rhythms in the suprachiasmatic nucleus [In Process Citation]. Prog Brain Res 119, 351-64 (1998).

84. Jin, X. et al. A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. Cell 96, 57-68 (1999).

85. Boer, G. J., van Esseveldt, K. E., van der Geest, B. A., Duindam, H. & Rietveld, W. J. Vasopressin-deficient suprachiasmatic nucleus grafts re-instate circadian rhythmicity in suprachiasmatic nucleus-lesioned arrhythmic rats. Neuroscience 89, 375-85 (1999).

86. Wideman, C. H., Murphy, H. M. & Nadzam, G. R. Vasopressin deficiency provides evidence for separate circadian oscillators of activity and temperature. Peptides 21, 811-6. (2000).

87. Jansen, K., Van der Zee, E. A. & Gerkema, M. P. Organotypic suprachiasmatic nuclei cultures of adult voles reflect locomotor behavior: differences in number of vasopressin cells. Chronobiol Int 16, 745-50 (1999).

88. Ouyang, Y. & Johnson, C. H. Are circadian clocks adaptive? Light Treatment and Biological Rhythms 10, 6-8 (1998).

89. Hurd, M. W. & Ralph, M. R. The significance of circadian organization for longevity in the golden hamster. J Biol Rhythms 13, 430-6 (1998).

90. Scheer, F. A. & Buijs, R. M. Light affects morning salivary cortisol in humans. J Clin Endocrinol Metab 84, 3395-8 (1999).

91. Penev, P. D., Kolker, D. E., Zee, P. C. & Turek, F. W. Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. Am J Physiol 275, H2334-7 (1998).

92. Verdecchia, P. et al. Adverse prognostic value of a blunted circadian rhythm of heart rate in essential hypertension. J Hypertens 16, 1335-43 (1998).

93. Knutsson, A., Akerstedt, T., Jonsson, B. G. & Orth-Gomer, K. Increased risk of ischaemic heart disease in shift workers. Lancet 2, 89-92 (1986).

94. Dumont, M., Montplaisir, J. & Infante-Rivard, C. Sleep Quality of Former Night-shift Workers. Int J Occup Environ Health 3, S10-S14 (1997).

95. Cho, K. Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. Nat Neurosci 4, 567-8. (2001).

96. Cho, K., Ennaceur, A., Cole, J. C. & Suh, C. K. Chronic jet lag produces cognitive deficits. J Neurosci 20, RC66 (2000).

97. Van Someren, E. J. Article reviewed: Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. Sleep Med 3, 81-2 (2002).

98. Fuller, C. A., Sulzman, F. M. & Moore-Ede, M. C. Thermoregulation is impaired in an environment without circadian time cues. Science 199, 794-6 (1978).

99. Boivin, D. B. et al. Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. Arch Gen Psychiatry 54, 145-52 (1997).

100. Monk, T. H. in Principles and Practice of Sleep Medicine (eds. Kryger, M. H., Roth, T. & Dement, W. C.) 321-330 (W.B. Saunders, Philadelphia, 1994).

101. Avery, D. H., Shah, S. H., Eder, D. N. & Wildschiodtz, G. Nocturnal sweating and temperature in depression. Acta Psychiatr Scand 100, 295-301 (1999).

102. Deuschle, M. et al. Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. J Clin Endocrinol Metab 82, 234-8 (1997).

103. Brown, R. P. et al. Depressed mood and reality disturbance correlate with decreased nocturnal melatonin in depressed patients. Acta Psychiatr Scand 76, 272-5 (1987).

104. Miles, A. & Philbrick, D. R. Melatonin and psychiatry. Biol Psychiatry 23, 405-25 (1988).

105. Souetre, E. et al. Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality. Psychiatry Res 28, 263-78 (1989).

106. Zhou, J. N. et al. Alterations in arginine vasopressin neurons in the suprachiasmatic nucleus in depression. Arch Gen Psychiatry 58, 655-62 (2001).

107. Kalsbeek, A., Buijs, R. M., van Heerikhuize, J. J., Arts, M. & van der Woude, T. P. Vasopressin-containing neurons of the suprachiasmatic nuclei inhibit corticosterone release. Brain Res 580, 62-7 (1992).

108. Morin, L. P. Serotonin and the regulation of mammalian circadian rhythmicity. Ann Med 31, 12-33 (1999).

109. Jewett, M. E. & Kronauer, R. E. Interactive mathematical models of subjective alertness and cognitive throughput in humans. J Biol Rhythms 14, 588-97 (1999).

110. Romani, A. et al. Circadian and hypothermia-induced effects on visual and auditory evoked potentials in multiple sclerosis. Clin Neurophysiol 111, 1602-6 (2000).

111. Wyatt, J. K., Ritz-de Cecco, A., Czeisler, C. A. & Dijk, D.-J. Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. Am. J. Physiol. 277, R1152-R1163 (1999).

112. Aggleton, J. P., Blindt, H. S. & Candy, J. M. Working memory in aged rats. Behav Neurosci 103, 975-83 (1989).

113. Bonnet, M. H. The effect of sleep fragmentation on sleep and performance in younger and older subjects. Neurobiol Aging 10, 21-5 (1989).

114. Devan, B. D. et al. Circadian phase-shifted rats show normal acquisition but impaired long-term retention of place information in the water task. Neurobiol Learn Mem 75, 51-62 (2001).

115. Downey, R. & Bonnet, M. H. Performance during frequent sleep disruption. Sleep 10, 354-63 (1987).

116. Fekete, M., van Ree, J. M., Niesink, R. J. & de Wied, D. Disrupting circadian rhythms in rats induces retrograde amnesia. Physiol Behav 34, 883-7 (1985).

117. Holloway, F. A. & Wansley, R. A. Multiple retention deficits at periodic intervals after active and passive avoidance learning. Behav Biol 9, 1-14 (1973).

118. Oler, J. A. & Markus, E. J. Age-related deficits on the radial maze and in fear conditioning: hippocampal processing and consolidation. Hippocampus 8, 402-15 (1998).

119. Sandyk, R., Anninos, P. A. & Tsagas, N. Age-related disruption of circadian rhythms: possible relationship to memory impairment and implications for therapy with magnetic fields. Int J Neurosci 59, 259-62 (1991).

120. Stone, W. S. Sleep and aging in animals. Relationships with circadian rhythms and memory. Clin Geriatr Med 5, 363-79 (1989).

121. Tapp, W. N. & Holloway, F. A. Phase shifting circadian rhythms produces retrograde amnesia. Science 211, 1056-8 (1981).

122. Antoniadis, E. A., Ko, C. H., Ralph, M. R. & McDonald, R. J. Circadian rhythms, aging and memory. Behav Brain Res 111, 25-37 (2000).

123. Gais, S., Plihal, W., Wagner, U. & Born, J. Early sleep triggers memory for early visual discrimination skills. Nat Neurosci 3, 1335-9. (2000).

124. Graves, L., Pack, A. & Abel, T. Sleep and memory: a molecular perspective. Trends Neurosci 24, 237-43 (2001).

125. Mazzoni, G. et al. Word recall correlates with sleep cycles in elderly subjects. J Sleep Res 8, 185-8 (1999).

126. Stickgold, R., James, L. & Hobson, J. A. Visual discrimination learning requires sleep after training. Nat Neurosci 3, 1237-8 (2000).

127. Wyss, J. M., Swanson, L. W. & Cowan, W. M. A study of subcortical afferents to the hippocampal formation in the rat. Neuroscience 4, 463-76 (1979).

128. Takeda, T. Senescence-accelerated mouse (SAM): a biogerontological resource in aging research. Neurobiol Aging 20, 105-10 (1999).

129. Cohen, R. A. & Albers, H. E. Disruption of human circadian and cognitive regulation following a discrete hypothalamic lesion: a case study. Neurology 41, 726-9 (1991).

130. Sapolsky, R. M. Depression, antidepressants, and the shrinking hippocampus. Proc Natl Acad Sci U S A 98, 12320-2 (2001).

131. Lucassen, P. J. et al. Hippocampal apoptosis in major depression is a minor event and absent from subareas at risk for glucocorticoid overexposure. Am J Pathol 158, 453-68. (2001).

132. Muller, M. B. et al. Neither major depression nor glucocorticoid treatment affects the cellular integrity of the human hippocampus. Eur J Neurosci 14, 1603-1612 (2001).

133. Hajak, G. & Rodenbeck, A. Melatonin and cortisol rhythms in disturbed sleep. J Sleep Res 7, 112 (1998).

134. Rodenbeck, A. & Hajak, G. Increased evening cortisol levels and nocturnal awakenings in primary insomnia. J Sleep Res 7, 227 (1998).

135. Gonzalez Garcia-Portilla, P., Pedregal Sanchez, J. A., Lopez Rodriguez, J. L., Bousono Garcia, M. & Bobes Garcia, J. Sleep, organic deterioration and general health in the aging population. Luso Esp. Neurol. Psiquiatr. Cienc. Afines 19, 191-199 (1991).

136. Meguro, K. et al. Sleep disturbance in elderly patients with cognitive impairment, decreased daily activity and periventricular white matter lesions. Sleep 18, 109-14 (1995).

137. Meguro, K. et al. Disturbance in daily sleep/wake patterns in patients with cognitive impairment and decreased daily activity. J Am Geriatr Soc 38, 1176-82 (1990).

138. Bianchetti, A. et al. Predictors of mortality and institutionalization in Alzheimer disease patients 1 year after discharge from an Alzheimer dementia unit. Dementia 6, 108-112 (1995).

139. Moe, K. E., Vitiello, M. V., Larsen, L. H. & Prinz, P. N. Symposium: Cognitive processes and sleep disturbances: Sleep/wake patterns in Alzheimer's disease: relationships with cognition and function. J Sleep Res 4, 15-20 (1995).

140. Mortimer, J. A., Ebbitt, B., Jun, S. P. & Finch, M. D. Predictors of cognitive and functional progression in patients with probable Alzheimer's disease. Neurology 42, 1689-96 (1992).

141. Swaab, D. F. Brain aging and Alzheimer's disease, "wear and tear" versus "use it or lose it". Neurobiol Aging 12, 317-24 (1991).

142. Rutishauser, U. & Landmesser, L. Polysialic acid in the vertebrate nervous system: a promoter of plasticity in cell-cell interactions. Trends Neurosci 19, 422-7 (1996).

143. Campbell, S. S. & Murphy, P. J. Extraocular circadian phototransduction in humans. Science 279, 396-9 (1998).

144. Hebert, M., Martin, S. K. & Eastman, C. I. Nocturnal melatonin secretion is not suppressed by light exposure behind the knee in humans. Neurosci Lett 274, 127-30 (1999).

145. Lockley, S. W. et al. Extraocular light exposure does not suppress plasma melatonin in humans. Journal of Clinical Endocrinology and Metabolism 83, 3369-3372 (1998).

146. Jean-Louis, G., Kripke, D. F., Cole, R. J. & Elliot, J. A. No melatonin suppression by illumination of popliteal fossae or eyelids. J Biol Rhythms 15, 265-9 (2000).

147. Eastman, C. I., Martin, S. K. & Hebert, M. Failure of extraocular light to facilitate circadian rhythm reentrainment in humans.[In Process Citation]. Chronobiol Int 17, 807-26 (2000).

148. Koorengevel, K. M. et al. Extraocular light therapy in winter depression: a double-blind placebo-controlled study. Biol Psychiatry 50, 691-8 (2001).

149. Barinaga, M. Circadian clock. How the brain's clock gets daily enlightenment. Science 295, 955-7 (2002).

150. Berson, D. M., Dunn, F. A. & Takao, M. Phototransduction by retinal ganglion cells that set the circadian clock. Science 295, 1070-3 (2002).

151. Gooley, J. J., Lu, J., Chou, T. C., Scammell, T. E. & Saper, C. B. Melanopsin in cells of origin of the retinohypothalamic tract. Nat Neurosci 4, 1165 (2001).

152. Hannibal, J., Hindersson, P., Knudsen, S. M., Georg, B. & Fahrenkrug, J. The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. J Neurosci 22, RC191. (2002).

153. Hattar, S., Liao, H. W., Takao, M., Berson, D. M. & Yau, K. W. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295, 1065-70 (2002).

154. Provencio, I. et al. A novel human opsin in the inner retina. J Neurosci 20, 600-5 (2000).

155. Provencio, I., Rollag, M. D. & Castrucci, A. M. Photoreceptive net in the mammalian retina. Nature 415, 493 (2002).

156. Thapan, K., Arendt, J. & Skene, D. J. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. J Physiol 535, 261-7 (2001).

157. Hankins, M. W. & Lucas, R. J. The primary visual pathway in humans is regulated according to long-term light exposure through the action of a nonclassical photopigment. Curr Biol 12, 191-8 (2002).

158. Rea, M. A. Photic entrainment of circadian rhythms in rodents. Chronobiol Int 15, 395-423 (1998).

159. Meijer, J. H., Watanabe, K., Detari, L. & Schaap, J. Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats. Brain Res 741, 352-5 (1996).

160. Meijer, J. H., Watanabe, K., Schaap, J., Albus, H. & Detari, L. Light responsiveness of the suprachiasmatic nucleus: long-term multiunit and single-unit recordings in freely moving rats. J Neurosci 18, 9078-87 (1998).

161. Akiyama, M. et al. Inhibition of light- or glutamate-induced mPer1 expression represses the phase shifts into the mouse circadian locomotor and suprachiasmatic firing rhythms. J Neurosci 19, 1115-21 (1999).

162. Mikkelsen, J. D. A neuronal projection from the lateral geniculate nucleus to the lateral hypothalamus of the rat demonstrated with Phaseolus vulgaris leucoagglutinin tracing. Neurosci Lett 116, 58-63 (1990).

163. Johnson, R. F., Morin, L. P. & Moore, R. Y. Retinohypothalamic projections in the hamster and rat demonstrated using cholera toxin. Brain Res 462, 301-12 (1988).

164. Speh, J. C. & Moore, R. Y. Retinohypothalamic tract development in the hamster and rat. Brain Res Dev Brain Res 76, 171-81 (1993).

165. Chemelli, R. M. et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98, 437-51 (1999).

166. Costa, M. S. et al. Retinohypothalamic projections in the common marmoset (Callithrix jacchus): A study using cholera toxin subunit B. J Comp Neurol 415, 393-403 (1999).

167. Lu, J., Shiromani, P. & Saper, C. B. Retinal input to the sleep-active ventrolateral preoptic nucleus in the rat. Neuroscience 93, 209-14 (1999).

168. Youngstrom, T. G., Weiss, M. L. & Nunez, A. A. Retinofugal projections to the hypothalamus, anterior thalamus and basal forebrain in hamsters. Brain Res Bull 26, 403-11 (1991).

169. Bina, K. G., Rusak, B. & Semba, K. Localization of cholinergic neurons in the forebrain and brainstem that project to the suprachiasmatic nucleus of the hypothalamus in rat. J Comp Neurol 335, 295-307 (1993).

170. Murakami, N., Takahashi, K. & Kawashima, K. Effect of light on the acetylcholine concentrations of the suprachiasmatic nucleus in the rat. Brain Res 311, 358-60 (1984).

171. Zatz, M. & Brownstein, M. J. Intraventricular carbachol mimics the effects of light on the circadian rhythm in the rat pineal gland. Science 203, 358-61 (1979).

172. Moga, M. M., Weis, R. P. & Moore, R. Y. Efferent projections of the paraventricular thalamic nucleus in the rat. J Comp Neurol 359, 221-38 (1995).

173. Leander, P., Vrang, N. & Moller, M. Neuronal projections from the mesencephalic raphe nuclear complex to the suprachiasmatic nucleus and the deep pineal gland of the golden hamster (Mesocricetus auratus). J Comp Neurol 399, 73-93 (1998).

174. Shen, H. & Semba, K. A direct retinal projection to the dorsal raphe nucleus in the rat. Brain Res 635, 159-68 (1994).

175. Schaechter, J. D. & Sadun, A. A. A second hypothalamic nucleus receiving retinal input in man: the paraventricular nucleus. Brain Res 340, 243-50 (1985).

176. Francis, A. J. & Coleman, G. J. Phase response curves to ambient temperature pulses in rats. Physiol Behav 62, 1211-7 (1997).

177. Waterhouse, J. et al. Light of domestic intensity produces phase shifts of the circadian oscillator in humans. Neurosci Lett 245, 97-100 (1998).

178. Shimizu, T. et al. Dose-response relationship between the light intensity and muscle sympathetic nerve activity in man is very similar to that of circadian phase shift by light. Journal of Sleep Research 7, 247 (1998).

179. Hashimoto, S. et al. Midday exposure to bright light changes the circadian organization of plasma melatonin rhythm in humans. Neurosci Lett 221, 89-92 (1997).

180. Mishima, K., Okawa, M., Shimizu, T. & Hishikawa, Y. Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. J Clin Endocrinol Metab 86, 129-34 (2001).

181. Van Someren, E. J., Kessler, A., Mirmiran, M. & Swaab, D. F. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biol Psychiatry 41, 955-63 (1997).

182. Song, X. & Rusak, B. Acute effects of light on body temperature and activity in Syrian hamsters: influence of circadian phase. Am J Physiol Regul Integr Comp Physiol 278, R1369-80 (2000).

183. Badia, P., Myers, B., Boecker, M., Culpepper, J. & Harsh, J. R. Bright light effects on body temperature, alertness, EEG and behavior. Physiol Behav 50, 583-8 (1991).

184. Aizawa, S. & Tokura, H. The effects of different light intensities during the daytime on the forearm blood flow and mean body temperature in the evening. Jpn J Physiol 46, 481-4 (1996).

185. Aizawa, S. & Tokura, H. Exposure to bright light for several hours during the daytime lowers tympanic temperature. Int J Biometeorol 41, 90-3 (1997).

186. Krauchi, K., Cajochen, C., Danilenko, K. V. & Wirz-Justice, A. The hypothermic effect of late evening melatonin does not block the phase delay induced by concurrent bright light in human subjects. Neurosci Lett 232, 57-61 (1997).

187. Strassman, R. J., Qualls, C. R., Lisansky, E. J. & Peake, G. T. Elevated rectal temperature produced by all-night bright light is reversed by melatonin infusion in men. J Appl Physiol 71, 2178-82 (1991).

188. Campbell, S. S., Kripke, D. F., Gillin, J. C. & Hrubovcak, J. C. Exposure to light in healthy elderly subjects and Alzheimer's patients. Physiol Behav 42, 141-4 (1988).

189. Savides, T. J., Messin, S., Senger, C. & Kripke, D. F. Natural light exposure of young adults. Physiol Behav 38, 571-4 (1986).

190. Sanchez, R., Ge, Y. & Zee, P. C. A comparison of the strength of external Zeigebers in young and older adults. Sleep Research 22, 416 (1993).

191. Shochat, T., Martin, J., Marler, M. & Ancoli-Israel, S. Illumination levels in nursing home patients: effects on sleep and acitvity rhythms. J Sleep Res 9, 373-379 (2000).

192. Espiritu, R. C. et al. Low illumination experienced by San Diego adults: association with atypical depressive symptoms. Biol Psychiatry 35, 403-7 (1994).

193. Morita, T. & Tokura, H. The influence of different wavelengths of light on human biological rhythms. Appl Human Sci 17, 91-6 (1998).

194. Hughes, P. C. & Neer, R. M. Lighting for the elderly: a psychobiological approach to lighting. Hum Factors 23, 65-85 (1981).

195. Teresi, J., Lawton, M. P., Ory, M. & Holmes, D. Measurement issues in chronic care populations: dementia special care. Alzheimer Dis Assoc Disord 8 Suppl 1, S144-83 (1994).

196. Curcio, C. A. & Drucker, D. N. Retinal ganglion cells in Alzheimer's disease and aging. Ann Neurol 33, 248-57 (1993).

197. Curcio, C. A., Millican, C. L., Allen, K. A. & Kalina, R. E. Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. Invest Ophthalmol Vis Sci 34, 3278-96 (1993).

198. Blanks, J. C., Torigoe, Y., Hinton, D. R. & Blanks, R. H. Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. Neurobiol Aging 17, 377-84 (1996).

199. Hinton, D. R., Sadun, A. A., Blanks, J. C. & Miller, C. A. Optic-nerve degeneration in Alzheimer's disease. N Engl J Med 315, 485-7. (1986).

200. Sadun, A. A. & Bassi, C. J. The visual system in Alzheimer's disease. Res Publ Assoc Res Nerv Ment Dis 67, 331-47 (1990).

201. Davies, D. C., McCoubrie, P., McDonald, B. & Jobst, K. A. Myelinated axon number in the optic nerve is unaffected by Alzheimer's disease. Br J Ophthalmol 79, 596-600 (1995).

202. Justino, L. et al. Neuroretinal funciton is normal in early dementia of the Alzheimer type. Neurobiology of Aging 22, 691-695 (2001).

203. Klein, B. E. et al. Prevalence of glaucoma. The Beaver Dam Eye Study. Ophthalmology 99, 1499-504 (1992).

204. Quillen, D. A. Common causes of vision loss in elderly patients. Am Fam Physician 60, 99-108 (1999).

205. Bayer, A. U., Keller, O. N., Ferrari, F. & Maag, K. P. Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. Am J Ophthalmol 133, 135-7. (2002).

206. Sloan, M. A. et al. Aging affects the ocular circadian pacemaker of Aplysia californica. J Biol Rhythms 14, 151-9 (1999).

207. Sutin, E. L., Dement, W. C., Heller, H. C. & Kilduff, T. S. Light-induced gene expression in the suprachiasmatic nucleus of young and aging rats. Neurobiol Aging 14, 441-6 (1993).

208. Aujard, F. et al. Artificially accelerated aging by shortened photoperiod alters early gene expression (Fos) in the suprachiasmatic nucleus and sulfatoxymelatonin excretion in a small primate, Microcebus murinus. Neuroscience 105, 403-12 (2001).

209. Sumova, A., Travnickova, Z., Peters, R., Schwartz, W. J. & Illnerova, H. The rat suprachiasmatic nucleus is a clock for all seasons. Proc Natl Acad Sci U S A 92, 7754-8 (1995).

210. Gibson, G. E., Peterson, C. & Jenden, D. J. Brain acetylcholine synthesis declines with senescence. Science 213, 674-6 (1981).

211. Smith, M. L. & Booze, R. M. Cholinergic and GABAergic neurons in the nucleus basalis region of young and aged rats. Neuroscience 67, 679-88 (1995).

212. van der Zee, E. A., Streefland, C., Strosberg, A. D., Schroder, H. & Luiten, P. G. Colocalization of muscarinic and nicotinic receptors in cholinoceptive neurons of the suprachiasmatic region in young and aged rats. Brain Res 542, 348-52 (1991).

213. Endo, Y., Shinohara, K., Fueta, Y. & Irie, M. Influences of cholinergic neurotoxin ethylcholine aziridinium ion on circadian rhythms in rats. Neurosci Res 41, 385-90 (2001).

214. Gillette, M. U. & Tischkau, S. A. Suprachiasmatic nucleus: the brain's circadian clock. Recent Prog Horm Res 54, 33-58; discussion 58-9 (1999).

215. Harper, D. G. et al. Differential circadian rhythm disturbances in men with Alzheimer disease and frontotemporal degeneration. Arch Gen Psychiatry 58, 353-60. (2001).

216. Volicer, L., Harper, D. G., Manning, B. C., Goldstein, R. & Satlin, A. Sundowning and circadian rhythms in Alzheimer's disease. Am J Psychiatry 158, 704-11 (2001).

217. Monk, T. H., Buysse, D. J., Carrier, J. & Kupfer, D. J. Inducing jet-lag in older people: directional asymmetry. J Sleep Res 9, 101-16 (2000).

218. Klerman, E. B., Duffy, J. F., Dijk, D. J. & Czeisler, C. A. Circadian phase resetting in older people by ocular bright light exposure. J Investig Med 49, 30-40 (2001).

219. Benloucif, S., L'Hermite-Baleriaux, M., Weintraub, S. & Zee, P. C. in Seventh Meeting of the Society of Research on Biological Rhythms 133 (Jacksonville, FL, 2000).

220. Moore, R. Y. & Lenn, N. J. A retinohypothalamic projection in the rat. J Comp Neurol 146, 1-14 (1972).

221. Pate, J. R. Trans-neural atrophy of the nucleus ovoideus following eye removal in cats. Anatomical Records 67, 39 (1937).

222. Brockhaus, H. J Psych Neurol (LPZ) 51, 96-196 (1942).

223. Duncan, M. J., Cheng, X. & Heller, K. S. Photoperiodic exposure and time of day modulate the expression of arginine vasopressin mRNA and vasoactive intestinal peptide mRNA in the suprachiasmatic nuclei of Siberian hamsters. Brain Res Mol Brain Res 32, 181-6 (1995).

224. Bobbert, A. C. & Riethoven, J. J. Feedback in the rabbit's central circadian system, revealed by the changes in its free-running food intake pattern induced by blinding, cervical sympathectomy, pinealectomy, and melatonin administration. J Biol Rhythms 6, 263-78 (1991).

225. Johnson, R. F., Moore, R. Y. & Morin, L. P. Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Res 460, 297-313 (1988).

226. Monk, T. H., Buysse, D. J., Billy, B. D., Kennedy, K. S. & Kupfer, D. J. The effects on human sleep and circadian rhythms of 17 days of continuous bedrest in the absence of daylight. Sleep 20, 858-64 (1997).

227. Tabandeh, H. et al. Disturbance of sleep in blindness. Am J Ophthalmol 126, 707-12 (1998).

228. Kendall, A. R., Lewy, A. J. & Sack, R. L. Effects of aging on the intrinsic circadian period of totally blind humans. J Biol Rhythms 16, 87-95 (2001).

229. Husby, R. & Lingjaerde, O. Prevalence of reported sleeplessness in northern Norway in relation to sex, age and season. Acta Psychiatr Scand 81, 542-7 (1990).

230. Gordo, M. A., Recio, J. & Sanchez-Barcelo, E. J. Decreased sleep quality in patients suffering from retinitis pigmentosa. J Sleep Res 10, 159-64 (2001).

231. Ionescu, D., Driver, H. S., Heon, E., Flanagan, J. & Shapiro, C. M. Sleep and daytime sleepiness in retinitis pigmentosa patients. J Sleep Res 10, 329-35 (2001).

232. Depres-Brummer, P., Levi, F., Metzger, G. & Touitou, Y. Light-induced suppression of the rat circadian system. Am J Physiol 268, R1111-6 (1995).

233. Morgan, J. L. M., Graber, C. & Kirkby, J. D. Constant light and increased ambient termperature disrupt the circadian rhythm of body temperature in the domestic fowl (Gallus Domesticus). Soc Neuroscience Abstracts 23, 1323 (1997).

234. Usui, S., Takahashi, Y., Honda, Y. & Ebihara, S. Circadian behavioral rhythms during various light-intensity cycles in rats. Physiol Behav 46, 521-8 (1989).

235. Jean-Louis, G., Kripke, D. F. & Ancoli-Israel, S. Sleep and quality of well-being. Sleep 23, 1115-21 (2000).

236. Lucassen, P. J., Hofman, M. A. & Swaab, D. F. Increased light intensity prevents the age related loss of vasopressin- expressing neurons in the rat suprachiasmatic nucleus. Brain Res 693, 261-6 (1995).

237. Kohsaka, M. et al. Effects of moderately bright light on subjective evaluations in healthy elderly women. Psychiatry Clin Neurosci 53, 239-41 (1999).

238. Campbell, S. S. & Dawson, D. Bright light treatment of sleep disturbance in older subjects. Sleep Research 20, 448 (1991).

239. Kanikowska, D., Hirata, Y., Hyun, K. & Tokura, H. Acute phase proteins, body temperature and urinary melatonin under the influence of bright and dim light intensities during the daytime. J Physiol Anthropol Appl Human Sci 20, 333-8 (2001).

240. Rutkowska, D., Park, S. J., Okazaki, J. & Tokura, H. Effects of the different light intensities on some physiological parameters in terms of circadian rhythm. Applied Human Science 15, 129 (1996).

241. Weinert, D., Weiner, H. & Sturm, J. Internal and external desynchronization in old mice; effect of zeitgeber strength. Biological Rhythm Research 30, 279 (1999).

242. Park, S. J. & Tokura, H. Bright light exposure during the daytime affects circadian rhythms of urinary melatonin and salivary immunoglobulin A. Chronobiol Int 16, 359-71 (1999).

243. Kabuto, M., Otsuka, T., Saito, K. & Maruyama, S. A preliminary report on the levels and rhythmicity of urinary melatonin in the patients with senile dementia of the Alzheimer type. Mental Health Research 29, 65-77 (1982).

244. Nair, N. P., Hariharasubramanian, N., Pilapil, C., Isaac, I. & Thavundayil, J. X. Plasma melatonin rhythm in normal aging and Alzheimer's disease. J Neural Transm 21, 494 (1986).

245. Nair, N. P., Hariharasubramanian, N., Pilapil, C., Isaac, I. & Thavundayil, J. X. Plasma melatonin-- an index of brain aging in humans? Biol Psychiatry 21, 141-50 (1986).

246. Sack, R. L., Lewy, A. J., Erb, D. L., Vollmer, W. M. & Singer, C. M. Human melatonin production decreases with age. Journal of Pineal Research 3, 379-388 (1986).

247. Sharma, M. et al. Circadian rhythms of melatonin and cortisol in aging. Biol Psychiatry 25, 305-19 (1989).

248. Skene, D. J. et al. Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease. Brain Res 528, 170-4 (1990).

249. Uchida, K., Okamoto, N., Ohara, K. & Morita, Y. Daily rhythm of serum melatonin in patients with dementia of the degenerate type. Brain Res 717, 154-9 (1996).

250. Mishima, K. & Hishikawa, Y. in Sleep and Sleep Disorders: from Molecule to Behavior (eds. Hayaishi, O. & Inoue, S.) 177-191 (Academic Press, Kyoto, 1997).

251. Ohashi, Y. et al. Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzheimer's type. Biol Psychiatry 45, 1646-52 (1999).

252. Witting, W., Mirmiran, M., Bos, N. P. & Swaab, D. F. Effect of light intensity on diurnal sleep-wake distribution in young and old rats. Brain Res Bull 30, 157-62 (1993).

253. Fukuda, N. et al. Effects of short duration morning bright light in healthy elderly subjects. I: subjective feeling and ophthalmological examinations. Psychiatry Clin Neurosci 52, 250-1 (1998).

254. Campbell, S., Satlin, A., Volicer, L., Ross, V. & Herz, L. Management of behavioral and sleep disturbance in Alzheimer's patients using timed exporsure to bright light. Sleep Research 20, 446 (1991).

255. Kobayashi, R. et al. Effects of bright light at lunchtime on sleep of patients in a geriatric hospital I. Psychiatry Clin Neurosci 55, 287-9. (2001).

256. Bonnet, M. H. & Arand, D. L. Heart rate variability in insomniacs and matched normal sleepers. Psychosom Med 60, 610-5 (1998).

257. Okawa, M. et al. Sleep-wake rhythm disorders and their phototherapy in elderly patients with dementia. Jpn J Psychiatry Neurology 43, 293-295 (1989).

258. Dowling, G. A., Luxenberg, J. S., Davids, H. R., Davidson, A. & Hubbard, E. in Alzheimer Meeting (Washington, 2000).

259. Hozumi, S. et al. Phototherapy for elderly patients with dementia and sleep-wake rhythm disorders - a comparison between morning and evening light exposure. Jpn J Psychiatry Neurology 44, 813-814 (1990).

260. Lyketsos, C. G., Lindell Veiel, L., Baker, A. & Steele, C. A randomized, controlled trial of bright light therapy for agitated behaviors in dementia patients residing in long-term care. Int J Geriatr Psychiatry 14, 520-5 (1999).

261. Mishima, K. et al. Morning bright light therapy for sleep and behavior disorders in elderly patients with dementia. Acta Psychiatr Scand 89, 1-7 (1994).

262. Okawa, M., Hishikawa, Y., Hozumi, S. & Hori, H. in Biological Psychiatry (ed. Racagni, G.) 837-40 (Elsevier, Amsterdam, 1991).

263. Okawa, M. et al. Circadian rhythm disorders in sleep-waking and body temperature in elderly patients with dementia and their treatment. Sleep 14, 478-85 (1991).

264. Satlin, A., Volicer, L., Ross, V., Herz, L. & Campbell, S. Bright Light treatment of Behavioral and Sleep Disturbances in Patients with Alzheimer's Disease. Am J Psychiatry 149, 1028-1032 (1992).

265. Lovell, B. B., Ancoli-Israel, S. & Gevirtz, R. Effect of bright light treatment on agitated behavior in institutionalized elderly subjects. Psychiatry Res 57, 7-12 (1995).

266. Okumoto, Y., Koyama, E., Matsubara, H., Nakano, T. & Nakamura, R. Sleep improvement by light in a demented aged individual. Psychiatry Clin Neurosci 52, 194-6 (1998).

267. Abegg, A. & Wettstein, A. [Phototherapy of behavioral disorders as a sequela of disordered circadian rhythm in dementia in the elderly--difficulties in practical clinical applications]. Schweiz Arch Neurol Psychiatr 144, 63-80 (1993).

268. Colenda, C. C., Cohen, W., McCall, W. V. & Rosenquist, P. B. Phototherapy for patients with Alzheimer disease with disturbed sleep patterns: results of a community-based pilot study. Alzheimer Dis Assoc Disord 11, 175-8 (1997).

269. Fukuda, N. et al. Effects of bright light at lunchtime on sleep in patients in a geriatric hospital II. Psychiatry Clin Neurosci 55, 291-3. (2001).

270. Rosenthal, N. E. et al. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry 41, 72-80 (1984).

271. Kripke, D. F. Light treatment for nonseasonal depression: speed, efficacy, and combined treatment. J Affect Disord 49, 109-17 (1998).

272. Partonen, T. & Lonnqvist, J. Bright light improves vitality and alleviates distress in healthy people. J Affect Disord 57, 55-61 (2000).

273. Sörensen, S. & Brunnström, G. Quality of light and quality of life: an intervention study among older people. Lighting Research Technology 27, 113-118 (1995).

274. Hanger, M. A., Ancoli-Israel, S., Kripke, D. F., Jones, D. W. & Almendarez, L. Effects of phototherapy on depression in nursing home residents. Sleep Research 21, 88 (1992).

275. Sumaya, I. C., Rienzi, B. M., Deegan, J. F., 2nd & Moss, D. E. Bright light treatment decreases depression in institutionalized older adults: a placebo-controlled crossover study. J Gerontol A Biol Sci Med Sci 56, M356-60. (2001).

276. Ito, T., Yamadera, H., Ito, R. & Endo, S. [Effects of bright light on cognitive disturbances in Alzheimer-type dementia]. Nippon Ika Daigaku Zasshi 66, 229-38 (1999).

277. Okawa, M., Mishima, K., Hishikawa, Y., Hozumi, S. & Hori, H. in Sleep-Wakefulness (eds. Kumar, V. M., Mallick, H. N. & Nayar, U.) 128-132 (Wiley Eastern, New Delhi, 1993).

278. Murphy, P. J. & Campbell, S. S. Enhanced performance in elderly subjects following bright light treatment of sleep maintenance insomnia. J Sleep Res 5, 165-72 (1996).

279. Graf, A. et al. The effects of light therapy on mini-mental state examination scores in demented patients. Biol Psychiatry 50, 725-7 (2001).

280. Yamadera, H. et al. Effects of bright light on cognitive and sleep-wake (circadian) rhythm disturbances in Alzheimer-type dementia. Psychiatry Clin Neurosci 54, 352-3 (2000).

281. Campbell, S. S. et al. Light treatment for sleep disorders: consensus report. III. Alerting and activating effects. J Biol Rhythms 10, 129-32 (1995).

282. Gonze, D., Halloy, J. & Goldbeter, A. Robustness of circadian rhythms with respect to molecular noise. Proc Natl Acad Sci U S A 99, 673-8 (2002).

283. Aston-Jones, G., Chen, S., Zhu, Y. & Oshinsky, M. L. A neural circuit for circadian regulation of arousal. Nat Neurosci 4, 732-8. (2001).

Chapter 3

Melatonin rhythms, melatonin supplementation and sleep in old age

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Abstract

The circadian timing system (CTS) allows organisms on earth to synchronize internal rhythms to the environmental 24-hour light-dark cycle and anticipate the body on the forthcoming period of either activity or rest. The central pacemaker of the CTS is the hypothalamic suprachiasmatic nucleus (SCN) regulating most, if not all, circadian rhythms in the body. The plasticity of this system at old age is the subject of this review, with special regard to its major internal stimulus, the pineal hormone melatonin.

The circadian rhythm in melatonin production is regulated by the SCN and results in low daytime circulating levels of melatonin and an increase of circulating melatonin after darkness onset. This rhythm, like other circadian rhythms, is attenuated in elderly subjects. The relation between the age-related change in melatonin levels and sleep is discussed in this paper.

Based on the hypothesis that there is a relationship between the increased prevalence of sleep-disturbances and decreased melatonin levels in elderly, several studies have been performed to investigate the effect of exogenous melatonin supplementation on sleep in elderly and demented subjects. An overview of these findings is presented and the various results are discussed.

Introduction

All living organisms on earth are exposed to the daily environmental 24-hour light-dark cycle. The circadian timing system (CTS) is able to synchronize an organism's internal rhythm to that of the environment, to allow the body to anticipate the coming period and to function with maximum efficiency in the given environmental situation. In humans this means that the body is prepared to waken before the light period, and to exhibit optimum physical and mental performance during the day. In the evening the body gets ready for the resting period.

The central coordinating pacemaker of the CTS in mammals is located in the suprachiasmatic nucleus (SCN), situated bilaterally in the anterior hypothalamus, on top of the optic chiasm. The SCN regulates circadian rhythms in body temperature, hormone levels and rest-activity cycle. By receiving environmental stimuli, the so-called Zeitgebers, the SCN synchronizes these rhythms to the 24-hour environmental light-dark cycle. In the absence of Zeitgebers the rhythms generated by the SCN will deviate from the exact 24-hour cycle. The CTS is a flexible system that can adapt to a new light-dark regimen after crossing several time zones.

The functional plasticity of the CTS at old age is the subject of this review. We will focus on a major internal stimulus for the CTS, the pineal hormone melatonin, which is endogenously present as a circadian modulator, but may be supplemented exogenously as well. Other effective stimuli, like bright light, temperature, physical activity and transcutaneous nerve stimulation (TENS) have been reviewed previously [1-6].

In the framework of the question to what extent the CTS and its plasticity are maintained at high age, especially in humans, the following points are subsequently discussed (1) the organization of the CTS, including the pineal gland, (2) age-related changes of the CTS, especially in relation to melatonin synthesis in the pineal gland (3) functional implications of weak or disturbed circadian rhythms, (4) interactions of the pineal melatonin synthesis with drugs frequently used by elderly people and (5) the supplementation of melatonin and its consequences for the CTS.

A. The circadian timing system

A.1 Structure of the suprachiasmatic nucleus

The suprachiasmatic nucleus (SCN) is a bilateral structure that is the central pacemaker of the circadian timing system (CTS) and regulates most, if not all, circadian rhythms in the body. Within the SCN several

types of peptidergic neurons are found, such as vasopressin (AVP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and neurotensin (NT), that each have a specific distribution. The vasopressinergic subnucleus has a volume of 0.25 mm^3 per side. The synthesis of vasopressin in the SCN shows both a circadian and seasonal rhythm [7].

A.2 Input to the suprachiasmatic nucleus

From the inputs the SCN receives, environmental light is the most effective one and is of direct and indirect importance for the melatonin production and rhythm (see further in this paper). The SCN receives information about the environmental light-dark cycle by a direct projection through the retinohypothalamic tract (RHT) [8], for which glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP) are at present the most likely transmitters [9]. Photoperiodic information from the environment is mediated by melanopsin-containing retinal ganglion cells projecting via the RHT to the SCN [10] (see figure 1).

In addition to the retinohypothalamic input, the SCN also receives inputs from other hypothalamic nuclei, the raphe nuclei, locus coeruleus, limbic forebrain, and from the hormonal milieu [11].

A.3 Output of the suprachiasmatic nucleus innervating the pineal gland

Both the acute suppression of melatonin by environmental light and the circadian modulation of the melatonin level are mediated by the SCN. The SCN-Pineal pathway has been elegantly confirmed by the use of the transneural pseudorabies viral tracer (PRV) [12]. PRV was injected into the pineal gland and labeling was subsequently found in the superior cervical ganglion (SCG), the intermediolateral column of the upper thoracic cord (IML), the autonomic division of the paraventricular nucleus (PVN), and the SCN. The majority of labeled neurons was found in the dorsomedial position of the SCN. Confocal laser scanning microscopy showed SCN neurons to be double-labeled for PRV and AVP and PRV and VIP. Removal of the SCG resulted in complete absence of the tracer in the SCN – pineal pathway, but not in the pineal gland. Control of the circadian variation of sympathetic input to the pineal is mediated by two signals from the SCN to the PVN, most likely a continuous stimulatory glutaminergic input and a rhythmic inhibitory GABA-ergic input. Without SCN input, a basic stimulatory effect on the pineal gland is maintained by the PVN, which is normally suppressed by the SCN during the light period and enhanced by the SCN during the dark period [13]. The neurotransmitters involved in the pathway from the PVN to the IML, and further from the IML to the SCG, are still unknown [14]. The direct inhibiting effect of nocturnal ocular light on melatonin levels is a result of the inhibition of N-acetyltransferase (NAT) activity (see following section) in the pineal gland [15, 16]. In rats, this light-induced inhibition of nocturnal melatonin release is completely prevented by the administration of a GABA-antagonist to the hypothalamic projection areas of the SCN [14]. Within one hour, nocturnal light exposure results in a decrease of melatonin levels. The rate of decline corresponds to the half-life time of melatonin [16], which means that there is a direct effect of light on the production of melatonin. Figure 1 shows schematically the connections between environmental light, the SCN and the pineal gland. For further details about the output pathways of the mammalian suprachiasmatic nucleus we refer to the review of Kalsbeek and Buijs [17]. In humans, the light-induced inhibition of nocturnal melatonin release is fully dependent on light that falls on the eyes, and supposedly mediated by the consequent retinal signal that reaches the SCN. Since the publication of Campbell et al. in 1998 [18], in which it was stated that extra-ocular light, administered behind the knees, could phase-shift the melatonin rhythm, several researchers have tried to replicate this finding, but without success. Neither did anyone find a suppression of melatonin by extra-ocular light [19, 20].

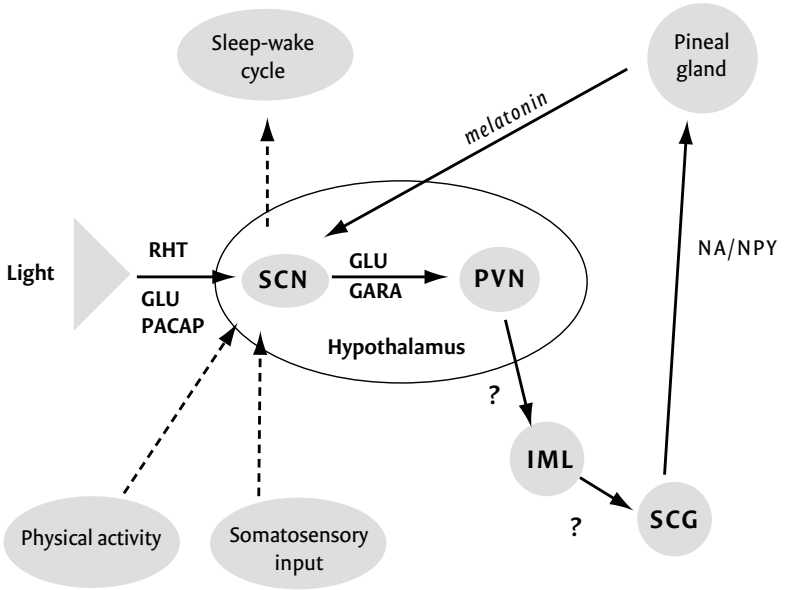

Figure 1
Schematic drawing of that part of the circadian timing system involved in the circadian regulation of melatonin
Abbreviations: RHT=retinohypothalamic tract, Glu=glutamate, PACAP= pituitary adenylate cyclase-activating polypeptide, SCN=suprachiasmatic nucleus, GABA=gamma-aminobutyric acid, PVN=paraventricular nucleus, SCG=superior cervical ganglion, NA=noradrenalin, NPY=neuropeptide Y

A.4 The pineal gland and its main hormone melatonin

This neuro-endocrine gland is present in many different species, among which all mammals. In humans the pineal gland is localized in the midline of the brain at the posterior level of the third ventricle. The hormone melatonin is the primary product of the pineal gland. Melatonin is synthesized of tryptophan (TRP) cf. [15]. The subsequent steps are as follows. First, tryptophan hydroxylase (TH) catalyzes the conversion from TRP into 5-hydroxytryptophan (5-HTP). The subsequent oxidation of 5-HTP into 5-HT (serotonin) is catalyzed by 5-HTP-decarboxylase. 5-HT is then N-acetylated by NAT into N-actyl-serotonin (NAS). NAT is considered the rate-limiting enzyme. Finally hydroxyindol-O-methyltransferase (HIOMT) catalyzes the O-methylation of NAS to form melatonin (N-acetyl-5-methoxytryptamine). These steps are summarized in figure 2.

The pineal gland is innervated by several neural pathways, as recently reviewed by Moller and Baeres cf. [21], among which the sympathetic fibers are the most important ones for the circadian regulation of melatonin production. These fibers contain noradrenalin and NPY cf. [21]. Noradrenalin, by stimulation of β-adrenergic receptors, leads to an increase in NAT cf. [22]. In addition to the sympathetic innervation via the superior cervical ganglion, by which the SCN regulates its circadian rhythm, the pineal gland is innervated by fibres of parasympathetic nerves, nerves from the trigeminal ganglion, and nerves originating from the brain, entering the pineal gland via the pineal stalk, also called the central innervation [21].

In both nocturnal and diurnal animals, the production of melatonin shows a circadian pattern with high levels after the onset

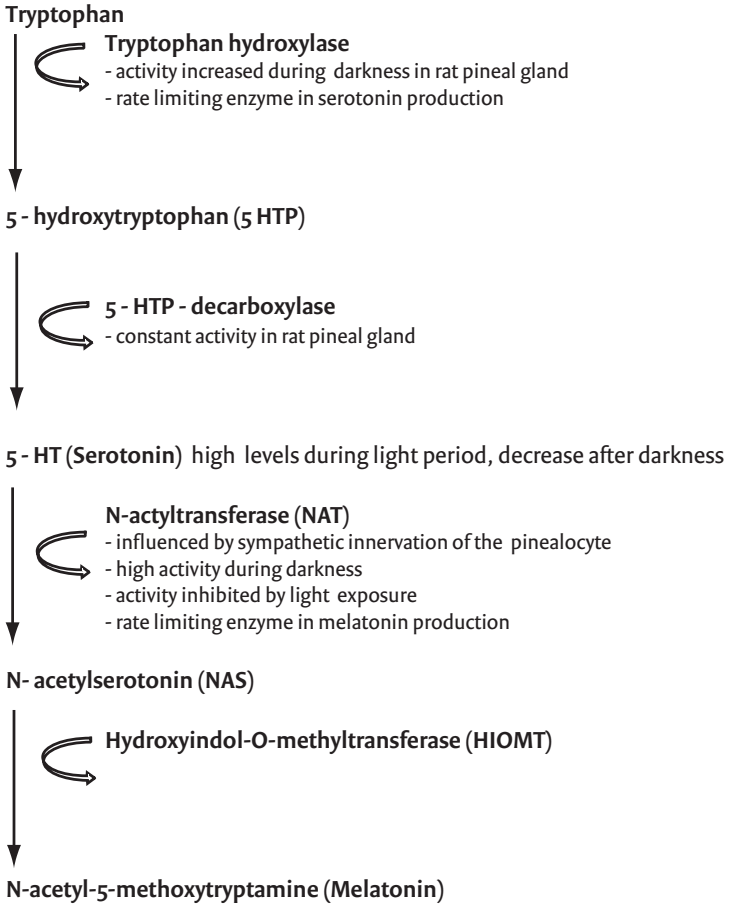

Figure 2
Subsequent steps of melatonin production in the pinealocyte

of darkness, and low levels during the light period of the day [23]. The circadian pattern in melatonin levels can be monitored directly, either in serum or saliva, or indirectly, by measuring its main metabolite 6-sulfatoxy melatonin in urine. Especially measurements in saliva are of interest for studies in elderly subjects and demented patients, and in field studies. Saliva sampling is relatively easy and non-invasive, and the correlation with plasma melatonin levels is strong [24]. The dim-light melatonin onset (DLMO) determines the time point at which melatonin starts to rise and rises above a certain threshold. This threshold can be defined in different ways. The DLMO for serum melatonin levels can be defined as the interpolated crossing of the 10 pg/ml threshold during its rise [25]. Other definitions use the time point at which the curve crosses 25% of the peak level [26, 27], or the time when the curve exceeds a level calculated from the mean plus two standard deviations of samples at 19:00, 19:30 and 20:00 h [28].

Its high lipophilicity allows melatonin to diffuse from the pineal into the surrounding tissue with ease and to enter into the bloodstream or the cerebrospinal fluid (CSF). For a long time it has been presumed that melatonin enters the CSF via the bloodstream. Recent studies in sheep, however, indicate that melatonin may also enter the CSF directly from the pineal gland [29].

A.5 Melatonin function and sites of action

Melatonin conveys photoperiodic information and thus informs the circadian timing system about the time of day and season cf. [15, 23].

Three pharmacologically distinct melatonin binding sites have been described, the MT-1, MT-2 and MT-3 receptors cf. [30], of which the MT-1 [31] and MT-2 are G-protein coupled receptors [32], and the MT-3 receptor [33] belongs to the family of quinone reductases. There is an abundant expression of melatonin receptors in the SCN [34]. In rat, the expression of MT-1 mRNA in the SCN exhibits a circadian variation [35], with the highest levels in the early day- and early nighttime periods. The specific binding of melatonin in the SCN of rat is significantly lower during the dark period compared to the light period. This difference disappears in constant darkness [35]. Melatonin decreases the frequency of the spontaneous electrical activity in cultured rat SCN explants cf. [36]

B. Age-related changes in the circadian timing system

B.1 Age-related changes in the suprachiasmatic nucleus

In post-mortem studies of the aging human SCN, the circadian rhythm that was found in young adults in the number of AVP-expressing neurons according to clock-time of death disappeared after the age of 50 years. Also the seasonal fluctuation in AVP-expressing neurons disappeared in the group of elderly subjects. Furthermore, a decline in the number of arginine vasopressin (AVP) expressing neurons was found in subjects over the age of 80 years and even more so, and at a younger age, in Alzheimer patients cf. [7]. There was also a decrease in the amount of AVP-mRNA in the SCN of Alzheimer patients [37]. The number of VIP-expressing neurons declined only in male subjects and not in female subjects [38].

B.2 Age-related changes in melatonin levels, amplitude and rhythmicity

Age-related changes have been demonstrated throughout the melatonin system, i.e. at the structural level in the pineal gland itself, in the total production level and in the rhythmicity of melatonin levels. Kunz et al. [39] described an increase of the calcified portion of the pineal and suggested the consequent decrease in uncalcified pineal tissue to be responsible for a decrease in aMT6s excretion levels. A post-mortem study showed that, by comparing the pineal glands of subjects that died young (age range 18-54 years) with those of subjects that died at old age (age range 55-92 years), the daily variation in melatonin levels disappears with aging [40]. The number of beta-adrenergic receptors in the pinealocyte membrane, important for the sympathetic innervation that regulates the circadian rhythmicity of the melatonin production, decreases with age, as does the responsiveness to norepinephrine [41].

A general finding in elderly people is that the nocturnal peak level is attenuated [42-50], resulting in a decrease in the amplitude of the melatonin rhythm (see table 1 for an overview of different studies on this topic). This decrease may occur very early in the process of development and aging. Kennaway [51] found the age-related decrease in urinary excretion of aMT6S, the main metabolite of melatonin, to occur already before the age of 30 years. Waldhauser et al. [52] also found the strongest decline of serum melatonin levels before the age of 20 years. No further decline was found between 20 and 70 years; only after the age of 70 years nighttime levels were lower compared to the younger adult groups. Zeitzer et al. [53] did not find an age-related decline in plasma melatonin levels. One reason for this could be that they included only extremely healthy people in their study. Another reason, suggested by Touitou [54], is the fact that Zeitzer et al. used a constant routine protocol, whereas in other studies subjects remained in their usual living environment. The importance of the illumination levels in the habitual environment has

been demonstrated by Mishima et al [55], who compared groups of young adults, elderly insomniacs and elderly people without sleep-complaints. They found significantly smaller amplitude of the melatonin rhythm in both groups of elderly subjects. An interesting finding then was that the supplementation of midday bright light (2500 lux) in the elderly insomniacs resulted in an increase of the melatonin amplitude to levels comparable with those in young adults [55]. Unfortunately this effect was not tested for the elderly subjects without sleep complaints. The importance of sufficient daytime bright light for the melatonin rhythm was also shown by Baskett et al. [56], who compared a group of hospitalized elderly with a community-based group of elderly. They found an attenuated daytime suppression of melatonin levels in the hospitalized group, together with more variable nighttime melatonin levels. Indeed, such an age-related increase of daytime melatonin level has often been reported in addition to the decrease in nocturnal level, as reviewed in Van Someren et al. [5]. In a study of Ohashi et al. [57], both demented and non-demented hospitalized psychiatric patients showed an increase of daytime melatonin levels compared to a group of non-hospitalized elderly subjects. Two hours of morning bright light (3000 lux) decreased daytime melatonin only in psychiatric non-demented elderly, but did not affect daytime melatonin in the demented group. In relation to this, the season might be an important variable to be taken into account when studying circadian rhythms. Whereas the decrease in amplitude is the most consistent finding on the melatonin rhythm in elderly, some studies have reported a change in the circadian phase of the melatonin rhythm. On this point the findings are less consistent: some studies reported a phase advance [46, 50, 58] while others found a phase delay [43, 48].

C. Functional implications of weak or disturbed circadian rhythms

An extensive review on the implications of weak or disturbed circadian rhythms was recently given by Van Someren [5]. Disturbed circadian rhythms appear to have negative effects on the impact of cardiovascular diseases, mood and cognition. In the present paper we will focus on the effect of changes in melatonin levels.

The relation between melatonin levels and sleep disturbances is not entirely clear. The knowledge that melatonin levels are decreased in elderly subjects, and that the prevalence of sleep disturbances is increased in this group [59], has led to the expectation that there would be a relation between melatonin levels and sleep. Although Haimov et al. [60] found a difference in melatonin levels between elderly insomniacs and elderly without sleep problems, others were unable to confirm this observation, either for melatonin levels, or for its metabolite aMT6S, and sleep parameters [61-64]. Although there is thus not a clear cut relationship between decreased melatonin levels and sleep, there might be a relation between sleep disturbances and the circadian period of the melatonin rhythm. Kripke et al. [61] reported that elderly volunteers with more deviant acrophases of aMT6S slept fewer hours and had more wake time within the sleep period.

D. Interactions with drugs frequently used by elderly

Several studies have addressed the influence of sympathetic agonists and antagonists on melatonin production. Hurlbut et al. [65], for example, investigated the influence of β-agonists and α- and β-antagonists on NAT and melatonin levels in Richardson's ground squirrels. They found that isoproterenol, a β-receptor agonist, stimulated both pineal NAT activity and pineal melatonin content; phen-

Table 1. Changes of melatonin levels with ageing

Research group	phase	peak level	mean	overall production	daytime melatonin level	inter-individual variability
Iguichi et al. 1982 [42]		decrease	decrease	decrease	decrease	
Sack et al. 1986 [47]				decrease		
Nair et al. 1986 [43]	delay	decrease		decrease		
Sharma et al. 1989 [48]	delay	decrease	decrease			
Touitou et al. 1984 [58]	advance	decrease	decrease	decrease	low	
Ferrari et al. 1993 [44]		decrease	decrease	decrease		
Mishima et al. 1994 [45]	no change	decrease	decrease	decrease	no change	
Zhdanova et al. 1998 [50]	advance	decrease				higher
Waldhauser et al. 1988 [52]		decrease				
Rodenbeck et al. 1998 [46]	advance	decrease				higher (according to the st. dev.)
Zeitzer et al. 1999 [53]		no change		no change		
Kennaway et al. 1999 [51]		decrease (until the age of 30)				

tolamine, an α-blocker, partially blocked the rise of NAT. Propranolol, a β-blocker, totally blocked this rise. Melatonin synthesis was not influenced by phentolamine, but administration of propranolol prevented the rise of melatonin during the night. This means that NAT activity is under the influence of both α and β innervation, while melatonin production is under the influence of β-innervation only.

The study of Stoschitzky et al. [66] is interesting in this context, because they investigated the influence of the beta-blocking (S)-enantiomers, and also the non-blocking (R)-enantiomers of propranolol and atenolol in humans. Enantiomers are two related molecules with a different conformation, so that one of them (the S-enantiomer) can bind to a receptor and thus act as an agonist, while the other (R-enantiomer) one cannot bind to the receptor because of its different conformation, and can thus act as a placebo. They found that the (S)-enantiomers decreased the nocturnal excretion of aMT6s, whereas the (R)-enantiomers had no effect. The use of enantiomers makes sure that the β-blocking effect is responsible for the decreased excretion of aMT6s, and thus melatonin production, rather than an unspecific effect of β-blockers.

The role of GABA is an important one. Especially since sleep disturbances are often treated with benzodiazepines, which bind to the GABA receptor. In the pineal, the release of GABA is triggered by noradrenalin, through α1-adrenergic receptors. The effects of GABA are generally inhibitory to the noradrenalin-induced NAT activity, and thus to melatonin production. There is also feedback from GABA to the sympathetic fibers. Pre-synaptically, GABA acts on type A and B receptors, which appear to have opposite functions, respectively facilitating and inhibiting noradrenalin release. The inhibitory function dominates. This circuit offers resistance to the passage of information to the pineal, leading to more balanced responses cf. [67].

GABA receptors have a number of binding sites for clinically important drugs. Benzodiazepines bind to the GABA-receptor complex and enhance its sensitivity for GABA. In the pineal, the acute and chronic administration of the benzodiazepine diazepam inhibits NAT activity in the rat pineal gland [68], and decreases the content of NAS and melatonin [69]. Chronic administration of diazepam resulted also in decreased nocturnal plasma levels of melatonin. Benzodiazepines may not only directly affect the melatonin rhythm by acting on the pineal innervation, but also by altering the level of activity and consequently the phase and period of the central pacemaker in the suprachiasmatic nucleus [70, 71] which is responsible for the melatonin rhythm.

E. Melatonin supplementation and consequences for the circadian timing system

E.1 Pharmacokinetics

Melatonin is quickly absorbed and quickly excreted. Metabolization takes place in the liver, where 70% is converted in 6-sulphatoxy melatonin (aMT6S). All metabolites are excreted by the kidneys. The half-life time of melatonin varies between 10 and 40 minutes cf. [15].

Fourtillan et al. [72] described the biological availability of D_7 melatonin (melatonin with seven hydrogen atoms replaced by seven deuterium atoms) administered intravenously (i.v.) or orally to twelve young healthy volunteers. Following i.v. administration of 23 µg D_7 melatonin, a large difference in peak levels was seen for males and females (mean peak level was 124.8 ± 32.8 pg/ml for males and 169.0 ± 29.6 pg/ml for females). After oral administration of 250 µg melatonin, melatonin was rapidly absorbed with a mean maximum absorption time of 23 minutes for both males and females. Here, the mean peak levels were almost three times higher for females than males (243.7 ± 124.6 pg/ml for males and 623.107 ± 575.1 pg/ml for females). The fraction of melatonin systemically absorbed ranged from 1 to 37% (mean values were 8.6 ± 3.6 % for males versus 16.8 ± 12.7 % for females). A low bioavailability can either be due to poor oral absorption, a large first pass effect, or a combination of the two.

In addition to the individual and sex-related differences in pharmacokinetics, Zhdanova [50] further reported an increased variance in serum melatonin levels in elderly subjects as compared to young adults after the ingestion of 0.3 mg melatonin.

E.2 Clinical studies on the effects of exogenous melatonin on sleep in the elderly

In table 2 an overview is presented of randomized placebo-controlled studies on the effect of melatonin on sleep, performed in elderly.

The two most recent studies, by Serfaty et al. [73] and Baskett et al. [64], did not show a therapeutic effect on actigraphically derived parameters of sleep quality, with the only exception that Baskett found a significant decrease in awakenings in a group of normal sleepers. Serfaty et al. investigated the effect of melatonin on sleep in demented elderly patients without further specification of a clinical diagnosis. Since dementia can be caused by various underlying diseases, this might be a very heterogeneous group of subjects, and a possible effect in a specific disease group might therefore have been missed. Another factor that could play a role in the negative results in the study of Serfaty et al. is the choice of the outcome measures. The actigraphical parameters tested in the study of Serfaty are the median total time asleep, median number of awakenings, and sleep efficiency. Van Someren [74] reported on the improved sensitivity to the effect of bright light therapy on rest-activity rhythms in Alzheimer patients, and demonstrated that the light-induced improvement in coupling of the rest-activity rhythm to the environmental Zeitgeber of bright light is better detected using nonparametric procedures. Worthwhile to mention in this respect is also the finding by Serfaty and co-workers [73] that the carers' reports of sleep problems in demented elderly were not consistent with the objective evidence as obtained by actigrapy. This means that data collected through logs kept by carers should be interpreted with caution.

Baskett et al. [64] looked at elderly insomniacs and normal sleeping elderly, as was also done by Haimov et al. [60] and Zhdanova et al.[63]. Beside differences in dosage, Baskett et al. studied a period of 4 weeks of active treatment, whereas both other studies looked for the effect of only 1 week of active treatment. To measure sleep quality, Haimov and Baskett both made use of actigraphy to measure sleep quality. Haimov found an improvement of sleep efficiency and activity level after 1 week of daily ingestion of 2 mg sustained release melatonin, and a shorter sleep latency after 1 week of daily ingestion of 2 mg fast release melatonin. Baskett found neither an improvement in sleep efficiency, nor in sleep latency after 4 weeks of daily administration of 5 mg melatonin. Zhdanova did show an improvement of sleep efficiency, using polysomnography to measure sleep quality. However, Hughes [27], also using PSG as the outcome measure, found no change in sleep-efficiency. Three studies reported an improvement in sleep latency [27, 75, 76] without improvement of total sleep time, except for Garfinkel [77], who studied elderly insomniacs using benzodiazepines. In a later study they reported on the ability of melatonin to facilitate discontinuation of benzodiazepine use. Andrade [78] found that melatonin in medically ill hospitalized patients improved their self-rated sleep quality. Objective parameters were not tested in this study.

In general, this overview shows that the various publications show distinct results on the effect of melatonin on sleep in elderly subjects. It is therefore hard to draw any definite conclusions at this moment.

E.3 Adverse effects and dosage

The studies mentioned in table 2 did not report any serious side-effects of melatonin. Only self-limiting pruritus [79], headache and excessive drowsiness [64] were reported.

Various dosages have been studied (see table 2). Zhdanova et al. [63], who studied 0.1 mg, 0.3 mg and 3 mg melatonin found an effect of all three dosages on sleep efficiency in elderly insomniacs, but the best effect was observed after the ingestion of 0.3 mg. This dosage resulted in physiological plasma levels of melatonin, whereas the ingestion of 3 mg

melatonin not only resulted in pharmacological plasma melatonin levels but also in a significant decrease in core body temperature.

Conclusive remarks

In spite of the effort that has been made in studying the efficacy of melatonin on sleep disturbances in the elderly, still no clear conclusions can be drawn.

Since multiple factors influence circadian rhythmicity, conditions that resulted in clear effects in a laboratory setting might be less effective in a natural setting, where the effect of melatonin is counteracted by other factors influencing the circadian timing system. It seems possible – and may be preferable - to stimulate the endogenous production of melatonin rather than to increase melatonin levels by the supplementation of exogenous melatonin. The studies of Mishima et al., Ohashi et al. and Baskett et al. [55-57] suggest that sufficient daytime bright light will increase night-time melatonin levels and attenuate day-time melatonin levels, and thus improve the circadian rhythmicity of circulating endogenous melatonin. This also means that the season is an important variable in studies regarding melatonin rhythmicity.

It is clear that changes in the circadian system occur with aging, both at the side of input from the environment as in the endogenous parameters that inform us about the functioning of the SCN. It is also clear that this system is still susceptible to an increased input of the appropriate stimuli and shows flexibility to changes according to the level of input of these stimuli.

Only Zeitzer et al. [53] did not show changes in the peak level of melatonin of a group of very healthy elderly. It would be interesting to know whether the few older subjects that still show a clear circadian rhythm in melatonin levels belong to the subgroup of successful aging, and if so, what the exact role of melatonin might be in the process of aging. In that case melatonin supplementation might also be considered as one of the strategies to influence the process of aging e.g. by acting as an anti-oxidant or immune stimulant [80].

As concluded by Olde Rikkert in 2001[81], there is the need for larger studies before widespread use of melatonin in sleep disorders can be advocated. These studies should have the power to distinguish patient characteristics as co-variates in the analysis of a therapeutic effect of melatonin, in order to see which subgroup of patients can be expected to show a positive effect of melatonin and which cannot. We hope to answer a number of these questions in the near future, when our 3.5-year follow-up study on the effect of bright light, melatonin or a combination of the two on sleep, mood, behavior, and cognition in demented elderly will be finished.

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Table 2. Randomized placebo-controlled studies on the effect of exogenous melatonin on circadian disturbances in the elderly

Research group	Number of subjects	Subject specification	Duration of active treatment	Dosage	Time of suppletion	Main outcome	Side effects
Garfinkel et al. 1995 [79]	12	Elderly insomniacs	Three weeks	2 mg controlled release	Two hours before desired bedtime	AG measured improvement of sleep efficiency and wake-after sleep onset period, trend to shorter sleep latency, no effect on total sleep time	Self limiting pruritus in one subject in both placebo and melatonin treated group
Haimov et al. 1995 [60]	51	Normal sleeping elderly (n=25) and elderly insomniacs (n=26)*	One week (each type of tablet)	2 mg sustained-release (S-r) or 2 mg fast-release (F-r)	Two hours before desired bedtime	AG measured improvement of sleep efficiency and activity level (S-r) or shorter sleep latency (F-r)	No side effects reported
Garfinkel et al. 1997 [75]	21	Benzodiazepine treated elderly insomniacs	Three weeks	2 mg controlled release	Two hours before desired bedtime	AG measured improvement of sleep latency, wake-after sleep onset period, total sleep time, fragmental index and number of awakenings	No side effects reported
Hughes et al. 1998 [27]	16	Elderly insomniacs	Two weeks	0.5 mg immediate release or 0.5 mg controlled-release	Thirty minutes before fixed bedtime and/or 4 hours after bedtime	PSG measured decrease of sleep-latency, no effect on PSG measured sleep efficiency or total sleep time and actigraphically measured parameters or subjective ratings of sleep quality	No side effects reported
Jean Louis et al. 1998 [76]	10	Elderly insomniacs with MCI	Ten days	6 mg	Two hours before bedtime	AG measured improvement of sleep latency, circadian amplitude, transition from sleep to wakefulness, no effect on total sleep time and wake time after sleep onset, improvement of mood and delayed recall	No side effects reported

Table 2. Continued

Garfinkel et al. 1999 [77]	34	Benzodiazepine treated elderly insomniacs	Six weeks	2 mg controlled release	Two hours before bedtime	Facilitation of discontinuation of benzodiazepine use	Headache in two subjects treated with melatonin and one treated with placebo
Andrade et al. 2001 [78]	33	Medically ill subjects	8-16 days	Flexible dose regimen	At night	Improvement of self rated time to fall asleep, sleep quality, sleep depth, freshness on awakening	No side effects reported
Zhdanova et al. 2001 [63]	30	Both normal sleeping elderly (n=15) and elderly insomniacs (n=15)	One week (each dosage)	0.1 mg, 0.3 mg or 3 mg	Half an hour before fixed bedtime	PSG measured increase of sleep-efficiency, with the best effect after ingestion of 0.3 mg in the insomniac group, no effect in normal sleepers	3 mg dose lowered significantly core body temperature
Serfaty et al. 2002 [73]	44	Demented elderly	Two weeks	6 mg slow-release	Usual bedtime	No therapeutic effect on either AG derived sleep-parameters nor subjective measures of sleep quality	No side effects reported
Baskett et al. 2003 [64]	40	Normal sleeping elderly (n=20) and elderly insomniacs (n=20)	Four weeks	5 mg	At bedtime	Lower number of awakenings in normal sleepers, further no therapeutic effect on AG measured parameters of sleep-quality	Excessive drowsiness in one person only in melatonin condition, in another person both in melatonin and placebo condition

Abbrviations: PSG=polysomnographically, AG=actigraphically, MCI=mild cognitive impairment
* Of the insomniacs 8 where living independently and 18 where institutionalized elderly

References

1. Van Someren, E. J., Kessler, A., Mirmiran, M. & Swaab, D. F. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biol Psychiatry 41, 955-63 (1997).

2. Van Someren, E. J., Lijzenga, C., Mirmiran, M. & Swaab, D. F. Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. J Biol Rhythms 12, 146-56 (1997).

3. Van Someren, E. J., Scherder, E. J. & Swaab, D. F. Transcutaneous electrical nerve stimulation (TENS) improves circadian rhythm disturbances in Alzheimer disease. Alzheimer Dis Assoc Disord 12, 114-8 (1998).

4. Van Someren, E. J., Raymann, R. J., Scherder, E. J., Daanen, H. A. & Swaab, D. F. Circadian and age-related modulation of thermoreception and temperature regulation: mechanisms and functional implications. Ageing Res Rev 1, 721-78 (2002).

5. Van Someren, E. J., Riemersma, R. F. & Swaab, D. F. Functional plasticity of the circadian timing system in old age: light exposure. Prog Brain Res 138, 205-31 (2002).

6. Van Someren, E. J. W. Thermosensitivity of the circadian timing system. Sleep and Circadian Rhythms 1, 55-64 (2003).

7. Swaab, D. F. in The autonomic nervous system part I: normal functions (ed. Appenzeller, O.) 467-521 (Elsevier, 1999).

8. Dai, J., Van der Vliet, J., Swaab, D. F. & Buijs, R. M. Human retinohypothalamic tract as revealed by in vitro postmortem tracing. J Comp Neurol 397, 357-70 (1998).

9. Hannibal, J. Neurotransmitters of the retino-hypothalamic tract. Cell Tissue Res 309, 73-88 (2002).

10. Brainard, G. C. et al. Human melatonin regulation is not mediated by the three cone photopic visual system. J Clin Endocrinol Metab 86, 433-6. (2001).

11. Moore, R. Y. Entrainment pathways and the functional organization of the circadian system. Prog Brain Res 111, 103-19 (1996).

12. Teclemariam-Mesbah, R., Ter Horst, G. J., Postema, F., Wortel, J. & Buijs, R. M. Anatomical demonstration of the suprachiasmatic nucleus-pineal pathway. J Comp Neurol 406, 171-82 (1999).

13. Perreau-Lenz, S. et al. Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. Eur J Neurosci 17, 221-8 (2003).

14. Kalsbeek, A., Cutrera, R. A., Van Heerikhuize, J. J., Van Der Vliet, J. & Buijs, R. M. GABA release from suprachiasmatic nucleus terminals is necessary for the light-induced inhibition of nocturnal melatonin release in the rat [In Process Citation]. Neuroscience 91, 453-61 (1999).

15. Reiter, R. J. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev 12, 151-80 (1991).

16. Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A. & Markey, S. P. Light suppresses melatonin secretion in humans. Science 210, 1267-9 (1980).

17. Kalsbeek, A. & Buijs, R. M. Output pathways of the mammalian suprachiasmatic nucleus: coding circadian time by transmitter selection and specific targeting. Cell Tissue Res 309, 109-18 (2002).

18. Campbell, S. S. & Murphy, P. J. Extraocular circadian phototransduction in humans. Science 279, 396-9 (1998).

19. Lockley, S. W. et al. Extraocular light exposure does not suppress plasma melatonin in humans. Journal of Clinical Endocrinology and Metabolism 83, 3369-3372 (1998).

20. Hebert, M., Martin, S. K. & Eastman, C. I. Nocturnal melatonin secretion is not suppressed by light exposure behind the knee in humans. Neurosci Lett 274, 127-30 (1999).

21. Moller, M. & Baeres, F. M. The anatomy and innervation of the mammalian pineal gland. Cell Tissue Res 309, 139-50 (2002).

22. Moore, R. Y. Neural control of the pineal gland. Behav Brain Res 73, 125-30 (1996).

23. Arendt, J. Mammalian Pineal Rhythms. Pineal Research Reviews 3, 161-213 (1985).

24. Gooneratne, N. S. et al. The validity and feasibility of saliva melatonin assessment in the elderly. J Pineal Res 34, 88-94 (2003).

25. Lewy, A. J., Cutler, N. L. & Sack, R. L. The endogenous Melatonin Profile as a Marker for Circadian Phase Position. Journal of Biological Rhythms 14, 227-236 (1999).

26. Koorengevel, K. M. et al. Extraocular light therapy in winter depression: a double-blind placebo-controlled study. Biol Psychiatry 50, 691-8 (2001).

27. Hughes, R. J., Sack, R. L. & Lewy, A. J. The role of melatonin and circadian phase in age-related sleep-maintenance insomnia: assessment in a clinical trial of melatonin replacement. Sleep 21, 52-68 (1998).

28. Voultsios, A., Kennaway, D. J. & Dawson, D. Salivary Melatonin as a Circadian Phase Marker: Validation and Comparison to Plasma Melatonin. Journal of Biological Rhythms 12, 457-466 (1997).

29. Tricoire, H., Malpaux, B. & Moller, M. Cellular lining of the sheep pineal recess studied by light-, transmission-, and scanning electron microscopy: Morphologic indications for a direct secretion of melatonin from the pineal gland to the cerebrospinal fluid. J Comp Neurol 456, 39-47 (2003).

30. Witt-Enderby, P. A., Bennett, J., Jarzynka, M. J., Firestine, S. & Melan, M. A. Melatonin receptors and their regulation: biochemical and structural mechanisms. Life Sci 72, 2183-98 (2003).

31. Reppert, S. M., Weaver, D. R. & Ebisawa, T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 13, 1177-85 (1994).

32. Reppert, S. M. et al. Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. Proc Natl Acad Sci U S A 92, 8734-8 (1995).

33. Nosjean, O. et al. Identification of the melatonin-binding site MT3 as the quinone reductase 2. J Biol Chem 275, 31311-7 (2000).

34. Dubocovich, M. L., Benloucif, S. & Masana, M. I. Melatonin receptors in the mammalian suprachiasmatic nucleus. Behav Brain Res 73, 141-7 (1996).

35. Poirel, V. J., Masson-Pevet, M., Pevet, P. & Gauer, F. MT1 melatonin receptor mRNA expression exhibits a circadian variation in the rat suprachiasmatic nuclei. Brain Res 946, 64-71 (2002).

36. Vanecek, J. Cellular mechanisms of melatonin action. Physiological Reviews 78, 687-721 (1998).

37. Liu, R. Y. et al. Decreased vasopressin gene expression in the biological clock of Alzheimer disease patients with and without depression. J Neuropathol Exp Neurol 59, 314-22 (2000).

38. Zhou, J. N., Hofman, M. A. & Swaab, D. F. VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. Neurobiol Aging 16, 571-6 (1995).

39. Kunz, D. et al. A new concept for melatonin deficit: on pineal calcification and melatonin excretion. Neuropsychopharmacology 21, 765-72 (1999).

40. Skene, D. J. et al. Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease. Brain Res 528, 170-4 (1990).

41. Greenberg, L. H. Regulation of brain adrenergic receptors during aging. Fed Proc 45, 55-9 (1986).

42. Iguichi, H., Kato, K. I. & Ibayashi, H. Age-dependent reduction in serum melatonin concentrations in healthy human subjects. J Clin Endocrinol Metab 55, 27-9. (1982).

43. Nair, N. P., Hariharasubramanian, N., Pilapil, C., Isaac, I. & Thavundayil, J. X. Plasma melatonin--an index of brain aging in humans? Biol Psychiatry 21, 141-50 (1986).

44. Ferrari, E. et al. in Melatonin and the pineal gland - From basic science to clinical application (eds. Touitou, Y., Arendt, J. & Pevet, P.) 379-382 (Elsevier Science Publishers B.V., 1993).

45. Mishima, K. et al. Morning bright light therapy for sleep and behavior disorders in elderly

patients with dementia. Acta Psychiatr Scand 89, 1-7 (1994).

46. Rodenbeck, A., Huether, G., Ruther, E. & Hajak, G. Altered circadian melatonin secretion patterns in relation to sleep in patients with chronic sleep-wake rhythm disorders. J Pineal Res 25, 201-10. (1998).

47. Sack, R. L., Lewy, A. J., Erb, D. L., Vollmer, W. M. & Singer, C. M. Human melatonin production decreases with age. Journal of Pineal Research 3, 379-388 (1986).

48. Sharma, M. et al. Circadian rhythms of melatonin and cortisol in aging. Biol Psychiatry 25, 305-19 (1989).

49. Touitou, Y. & Haus, E. Alterations with aging of the endocrine and neuroendocrine circadian system in humans. Chronobiology International 17, 369-390 (2000).

50. Zhdanova, I. V., Wurtman, R. J., Balcioglu, A., Kartashov, A. I. & Lynch, H. J. Endogenous Melatonin Levels and the Fate of Exogenous Melatonin: Age Effects. Journal of Gerontology 53A, B293-B298 (1998).

51. Kennaway, D. J. et al. Urinary 6-sulfatoxymelatonin excretion and aging: New results and a critical review of the literature. J. Pineal Res. 27, 210-220 (1999).

52. Waldhauser, F. et al. Alterations in nocturnal serum melatonin levels in humans with growth and aging. J Clin Endocrinol Metab 66, 648-52. (1988).

53. Zeitzer, J. M. et al. Do plasma melatonin concentrations decline with age? [In Process Citation]. Am J Med 107, 432-6 (1999).

54. Touitou, Y. Human aging and melatonin. Clinical relevance. Exp Gerontol 36, 1083-100 (2001).

55. Mishima, K., Okawa, M., Shimizu, T. & Hishikawa, Y. Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. J Clin Endocrinol Metab 86, 129-34 (2001).

56. Baskett, J. J., Cockrem, J. F. & Todd, M. A. Melatonin levels in hospitalized elderly patients: a comparison with community based volunteers. Age Ageing 20, 430-4 (1991).

57. Ohashi, Y. et al. Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzheimer's type. Biol Psychiatry 45, 1646-52 (1999).

58. Touitou, Y. et al. Patterns of plasma melatonin with ageing and mental condition: stability of nyctohemeral rhythms and differences in seasonal variations. Acta Endocrinologica 106, 145-51 (1984).

59. Bliwise, D. L. Sleep in normal aging and dementia. Sleep 16, 40-81 (1993).

60. Haimov, I. et al. Melatonin replacement therapy of elderly insomniacs. Sleep 18, 598-603. (1995).

61. Kripke, D. F., Elliot, J. A., Youngstedt, S. D. & Smith, J. S. Melatonin: marvel or marker? Ann Med 30, 81-7. (1998).

62. Baskett, J. J. et al. Melatonin in older people with age-related sleep maintenance problems: a comparison with age matched normal sleepers. Sleep 24, 418-24. (2001).

63. Zhdanova, I. V. et al. Melatonin treatment for age-related insomnia. J Clin Endocrinol Metab 86, 4727-30 (2001).

64. Baskett, J. J. et al. Does melatonin improve sleep in older people? A randomised crossover trial. Age Ageing 32, 164-170 (2003).

65. Hurlbut, E. C., King, T. S., Richardson, B. A. & Reiter, R. J. The effects of the light:dark cycle and sympathetically-active drugs on pineal N-acetyltransferase activity and melatonin content in the Richardson's ground squirrel, Spermophilus richardsonii. Prog Clin Biol Res 92, 45-56 (1982).

66. Stoschitzky, K. et al. Influence of beta-blockers on melatonin release. Eur J Clin Pharmacol 55, 111-5 (1999).

67. Drijfhout, W. J. in Faculty of Science 226 (Rijksuniversiteit Groningen, Groningen, 1996).

68. Djeridane, Y. & Touitou, Y. Chronic diazepam administration differentially affects melatonin synthesis in rat pineal and Harderian glands. Psychopharmacology (Berl) 154, 403-7 (2001).

69. Wakabayashi, H., Shimada, K. & Satoh, T. Effects of Diazepam Administration on Melatonin

Synthesis in the Rat Pineal Gland in vivo. Chem Pharm Bull 39, 2674-2676 (1991).

70. Joy, J. E., Losee-Olson, S. & Turek, F. W. Single injections of triazolam, a short-acting benzodiazepine, lengthen the period of the circadian activity rhythm in golden hamsters. Experientia 45, 152-4 (1989).

71. Johnson, R. F., Smale, L., Moore, R. Y. & Morin, L. P. Lateral geniculate lesions block circadian phase-shift responses to a benzodiazepine. Proc Natl Acad Sci U S A 85, 5301-4 (1988).

72. Fourtillan, J. B. et al. Bioavailability of melatonin in humans after day-time administration of D(7) melatonin. Biopharm Drug Dispos 21, 15-22 (2000).

73. Serfaty, M., Kennell-Webb, S., Warner, J., Blizard, R. & Raven, P. Double blind randomised placebo controlled trial of low dose melatonin for sleep disorders in dementia. Int J Geriatr Psychiatry 17, 1120-7 (2002).

74. Van Someren, E. J. et al. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int 16, 505-18 (1999).

75. Garfinkel, D., Laudon, M. & Zisapel, N. Improvement of sleep quality by controlled-release melatonin in benzodiazepine-treated elderly insomniacs. Archives of Gerontology & Geriatrics 24, 223-231 (1997).

76. Jean-Louis, G., Von Gizycki, H. & Zizi, F. Melatonin effects on sleep, mood, and cognition in elderly with mild cognitive impairment. J Pineal Res 25, 177-183 (1998).

77. Garfinkel, D., Zisapel, N., Wainstein, J. & Laudon, M. Facilitation of benzodiazepine discontinuation by melatonin: a new clinical approach. Arch Intern Med 159, 2456-60 (1999).

78. Andrade, C., Srihari, B. S., Reddy, K. P. & Chandramma, L. Melatonin in medically ill patients with insomnia: a double-blind, placebo-controlled study. J Clin Psychiatry 62, 41-5. (2001).

79. Garfinkel, D., Laudon, M., Nof, D. & Zisapel, N. Improvement of sleep quality in elderly people by controlled-release melatonin. Lancet 346, 541-4 (1995).

80. Yu, B. P. Approaches to anti-aging intervention: the promises and the uncertainties. Mech Ageing Dev 111, 73-87 (1999).

81. Olde Rikkert, M. G. & Rigaud, A. S. Melatonin in elderly patients with insomnia. A systematic review. Z Gerontol Geriatr 34, 491-7 (2001).

Descriptive studies

Chapter 4

Alterations in arginine vasopressin neurons in the suprachiasmatic nucleus in depression

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Abstract

Background: Circadian rhythm disturbances are frequently found in depressed subjects. Although it has been presumed that these disturbances may reflect a disorder of the circadian pacemaker, this has never been established. The suprachiasmatic nucleus (SCN) is the pacemaker of the circadian timing system in mammals and arginine vasopressin (AVP) is one of its major neuropeptides. As peptide content is often taken as a measure for activity, we hypothesized that a decreased number of AVP-immunoreactive (-IR) neurons and amount of AVP-mRNA would be present in the SCN of depressed subjects.
Methods: Brains of 11 subjects suffering from major depression (8 cases) and bipolar disorder (3 cases), and of 11 controls, matched for sex, age and clock-time of death, were collected. The number of AVP-IR neurons in the SCN was determined by means of a digitizer (Calcomp). The amount of AVP-mRNA expression in the SCN was quantified with the IBAS-KAT image analysis system.
Results: In depressed subjects, the number of AVP-IR neurons in the SCN was more than one and one-half times higher than in controls, while the total masked area of silvergrains, as an estimate of the amount of AVP-mRNA, was about one half that of controls.
Conclusions: Contrary to our hypothesis, an increase in the number of AVP-IR neurons in the SCN in depression was found, together with an expected decrease in AVP-mRNA. These findings suggest that in depressed patients both the synthesis and release of AVP in the SCN is reduced, resulting in an impaired functional ability. A disbalance between AVP production and transport needs further investigation in future studies.

Introduction

The suprachiasmatic nucleus (SCN) is the circadian pacemaker of the mammalian brain, generating and coordinating diurnal rhythms, e.g. sleep-wakefulness, body temperature and hormonal rhythms[1, 2]. Over the years, a variety of studies have pointed to the possible involvement of the circadian pacemaker in depression[3-5]. An argument in favour of this idea is that in the melancholic type of depression, patients feel worst in the morning and typically suffer from early morning awakenings[3-5]. In addition, a decrease in the amplitude of body temperature is consistently found in depressed patients[4, 5]. Furthermore, the successful treatment of seasonal affective disorder with light therapy[6, 7], and, to a lesser extent, also of patients with non-seasonal affective disorders[8], has led to the hypothesis that the effect of bright light on depression acts on the circadian pacemaker via the retinohypothalamic tract[3, 5, 7, 9]. Whether the observed disturbances of circadian rhythms in depression indeed reflect a disorder of the SCN has, however, so far not been established.

Another important hypothalamic structure that is involved in depression consists of the corticotrophin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVN). The increased number of neurons expressing CRH and the increased amount of CRH-mRNA are signs of strong activation of these neurons in depression[10, 11]. These findings are of particular interest because there are similarities between the signs and symptoms of major depression and the behavioural effects of centrally administered CRH in animals[12] and CRH overproduction in transgenic mice[13]. Furthermore, there is a functional relationship between arginine vasopressin (AVP) in the SCN and CRH in the PVN. AVP neurons from the SCN inhibit CRH neurons in the PVN of rat[14]. In this way, the SCN plays a key role in the circadian regulation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in circadian fluctuations of cortisol levels.

On the basis of these observations,

we hypothesised that the functional ability of the SCN in maintaining normal biological rhythms might be diminished in depression. Our hypothesis was that the number of AVP containing neurons and the amount of AVP-mRNA in the SCN of depressed subjects would be decreased.

Subjects and methods

Subjects

Brains of 11 depressed subjects were collected and matched with 11 controls for sex, age and clock time of death (Table I). Brain material of both depressed and control subjects was obtained from the Netherlands Brain Bank (NBB; coordinator Dr. R. Ravid). Within the framework of the NBB autopsies take place after informed consent is given by the donor and/or the next-of-kin for the following: a) performing a brain autopsy; b) the subsequent use of the tissue and fluids obtained for scientific research; c) permission to use the donor's medical history for research purposes. The charts of the control subjects did not report any psychiatric or neurological disease, except for subject C2b. The diagnosis was established by the physician in attendance and confirmed by WJGH, psychiatrist, after reviewing the chart. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria[15] were used for the diagnosis of depression and bipolar disorder, at any time during life. No relatives were interviewed to give additional information to the chart diagnosis. In case data were missing, an additional interview took place with the physician who treated the subject. In this procedure DSM-IV criteria for the presence, duration and severity of symptoms of either major depression or bipolar disorder, as well as the exclusion of other psychiatric and neurological disorders were systematically scored. Eight patients fulfilled the criteria for a major depression (MD) and three fulfilled the criteria for a bipolar disorder (BD) (Table I). Four MD subjects and two BD subjects suffered from their last episode just prior to death. In the two BD cases, this last episode was a manic episode. For detailed information on the time of the last episode see Table I. A complete overview of the psychiatric medication in the past and in the last month prior to death for both depressed and control subjects is given in Table I. The chart did not reveal any alcohol or drug abuse among depressed subjects or control subjects at the time of death, but no toxicology screens were performed. Microscopical examination of the liver of subject D11 showed micro abscesses and infiltration with neutrophilic and eosinophylic granulocytes. These signs could be compatible with drug intoxication. Potential cases and controls were excluded if not enough material was available to stain the complete SCN. For this reason three controls of the immunocytochemical study were replaced by three other controls for the in situ hybridization study (C2, C7 and C9, see Table I).

Immunocytochemistry and morphometry

For immunocytochemical analysis of AVP, 6 μm thick paraffin sections through the entire SCN were stained with an antibody against AVP. The immunocytochemical and morphometric procedures were performed as described extensively elsewhere[16-19]. Briefly, measurements of the vasopressinergic SCN area and the number of cell nuclei were performed unilateraly by means of a digitizer (Calcomp). The rostro-caudal axis was determined by staining every 25th section starting from the lamina terminalis and ending at the caudal end of the optic chiasm. The rostral and caudal borders of the SCN were assessed by staining every tenth section in the area and by determining the sections in which , respectively, the first and the last AVP cells were present. The volume of the SCN was determined by integrating all the area measurements of the SCN sections that contained immunocytochemically stained cells. The numerical cell density of AVP-IR neurons was estimated by counting the total number of nuclear profiles per unit area followed by a discrete 'unfolding' procedure which included the modification proposed

Table 1. Brain material of depressed and control subjects *†

Subject	Sex	Age /Age at onset, y	Brain Weight, g	PMD, h	Fix, d	Time at death	Month of death	Cause of death	No. of Episodes/ End of Last Episode ‡, No. of Months before death	Past suicide Attempts§	Psychiatric Medication taken	
											In the Last Month	In the Past
D1	M	51/41	1390	75	28	2 PM	November	Respiratory insufficiency, lung emphysema	8/7	No	LI, HAL, PHT¶⊥	BZD
C1	M	49	1254	22	33	3:10 PM	November	Sepsis, colon carcinoma			BZD¶⊥	
D2	F	55/≤40	1320	7	52	7:45 AM	November	Heart failure, urosepsis	2/Death	Yes	SSRI, BRO	MAP, BZD, TCA, PHT
C2a	F	50	1210	7	40	7 AM	January	Renal insufficiency, multiple myeloma				
C2b#	F	58	1221	7	28	7:15 AM	March	Postoperative coma after craniotomy				
D3	M	61/≤50	1424	41	35	4:40 AM	October	Pneumonia	1/144	No	PHT	TCA
C3	M	63	1250	10	32	5 AM	January	Pneumonia				
D4	M	63/12	1210	20	33	2:15 PM	March	Heart failure	>6/Death	No	HAL, BZD, PHT	LI
C4	M	78	1442	8	24	12:15PM	July	Cardiac arrhythmia				
D5	M	70/40	1500	44	28	7 PM	December	Heart failure	4/Death	No	SSRI, BZD, CLZ	
C5	M	70	1454	9	33	8 AM	February	Pneumonia, renal failure				
D6	M	71/53	975	16	38	4:15 AM	February	Cerebral ischemia, pneumonia	4/Death	Yes	None	BZD, MAO-I, TCA
C6	M	74	1317	8	60	1 PM	November	Heart failure, myocardial infarction				
D7	M	71/≤65	1109	14	26	10:30 PM	August	Respiratory insufficiency	≥2/21	No	LI, BZD, MAO-I, PHT	

Tabel 1. Continued

C7a	M	85	1400	16	44	4:50 PM	July	Chronic myelocytic leukemia				
C7b#	M	61	2200	14	64	9:22 PM	April	Esophagus carcinoma				
D8	F	72/54	1287	22	39	7 PM	January	Pneumonia	≥3/36	No	BZD¶	MAP
C8	F	63	1216	6	32	5:01 PM	September	Mammacarcinoma, euthanasia			BZD⊥	BZD
D9	F	72/53	1116	28	35	4:20 AM	April	Heart failure, septic shock, pyelonephritis	≥4/108	No	BZD	MIA, TCA
C9a	F	73	1344	8	34	9:10 AM	February	Septic shock, pneumonia				
C9b#	F	65	nd	7	28	1:45 AM	February	Respiratory insufficiency				BZD
D10	M	74/74	1444	57	35	5:05 PM	March	Strangulation (suicide)	1/Death	Yes	ZUC, SSRI, BZD	None
C10	M	78	1440	7	32	4 AM	September	Heart failure, lung embolism				
D11	F	80/60	1300	33	69	8 AM	December	Pneumonia	≥4/Death	No	LI, HAL, ZUC	TCA, BRO, PHT, BZD, CAR
C11	F	78	1135	6	32	8 AM	November	Respiratory insufficiency, lung carcinoma			BZD	BZD

*PMD indicates postmortem delay; Fix, fixation time; D, depressed subject, C, control subject; M, male; F, female; LI, lithium; HAL, haloperidol; PHT, phenotiazine; BZD, benzodiazepine; SSRI, selective serotonin reuptake inhibitors; BRO, bromperidol; MAP, maprotiline; TCA, tricyclic antidepressants; CLZ, clozapine; MAO-I, monoamine oxidase inhibitor; MIA, mianserin; ZUC, zuclopentixol; and CAR, carbamazepine; nd, not determined.

† All patients suffered from major depression, except D1, D4, and D11, who had bipolar disorder

‡ All last episodes were depressive, except for subjects D4 and D11, whose last episode was a manic episode

§ Subject D2 attempted suicide 1 month before death, D6 7 days before death, and D10 died of the attempt

¶ Also used corticosteroids

⊥ Also used morphine

Because not enough material was available, these subjects replaced the "a" subjects for in situ hybridization

by Cruz-Orive[20] and a correction for section thickness (6 μm, z-axis). All nuclear profiles within a rectangular grid in one of the oculars which corresponded to 38,000 μm^2 in the section were measured according to Gundersen[21]. The total number of AVP-IR neurons was computed by multiplying the numerical cell density with the corresponding volume of the AVP subnucleus.

In situ hybridization (ISH) and quantitative analysis

For ISH, three control subjects (C2, C7, C9) were replaced by other matched controls (Table I) because not enough material was left to stain the entire SCN. Hybridization was performed on every 50th section of the SCN. Sections were randomly divided over 2 hybridization assays of approximately 120 sections each. The AVP probe (hvp3) consisted of an oligomer of 48 nucleotides complementary to bases 411-458 of the human preprovasopressin precursor[22]. The specificity of the probe has been described previously[23, 24]. The probe was 3'-end labelled using terminal deoxynucleotidyl transferase (Boehringer Mannheim) and [α-^{35}S] dATP (NEN Life Sciences) as described earlier[24]. Tissue pre-treatments were performed mainly as previously described[24] except for the deproteination and delipidation. Deproteination was done in Proteinase-K (10μg/ml at 37°C) for 15 min instead of 30 min. Delipidation was performed in 0.1% Triton X-100 in PBS for 10 min and sections were washed in PBS without dehydration before hybridization. Each section was incubated with 68 μl hybridization solution containing approximately 1 x 10^6 cpm labelled probe. After overnight incubation at 42 °C, the sections were rinsed in 1 x SSC for 30 min at 50 °C, 2 x 30 min 0.1 x SSC at 50 °C, and 2 x 30 min 0.1 x SSC at room temperature. Sections were dehydrated at room temperature in 300 mM ammonium acetate (pH 5.5) / ethanol 100% at volume ratios 1:1, 3:7, 1:9 and 0:1. In order to check the autoradiographic signal, a β-max hyperfilm (Amersham, UK) was apposed and developed after 5 days. Subsequently, slides were dipped in photographic emulsion (NTB2 Kodak USA) at 42 °C, dried on a cool glass plate and stored in a light tight box at 4 °C. After 17 days, slides were developed for 2 min in Dektol Developer (Sigma) at 15 °C and fixed in Kodak fixer (Sigma) at 15 °C for 10 min. Sections were washed to remove the fixative and counter-stained with thionin.

For quantitative analysis of the ISH signal of the AVP-mRNA in the SCN, an IBAS-KAT image analysis system was connected to a Bosch TYK9B TV camera equipped with a chalnycon tube mounted on a Zeiss microscope. The microscope was equipped with planapo objectives, a blue filter and a scanning stage. The main principle and procedure of the IBAS measurement have been extensively described before[25]. Briefly, the area of the SCN was manually outlined at low magnification (4x objective) and a grid of fields was superimposed. From this grid 50% of the fields indicated in red rectangles were randomly selected and stored (Fig. 1-A). Then, at high magnification (40x objective), each field was retrieved on the image analysis monitor (Fig. 1-B). A mask was superimposed over the silver grains in these images. After removing the blue filter the profiles identified as cells by means of thionin staining were manually outlined. Finally, (i) the total number of profiles expressing AVP-mRNA in the SCN and (ii) total mask area of the silver grains in the profiles were calculated as an estimate of total amount of AVP-mRNA in the SCN. In addition, total mask of the silver grains was divided by the total number of profiles in order to estimate the mean amount of AVP-mRNA per profile. This gives an estimate of the average AVP-RNA production per neuron.

Neither for the assessment of the number of AVP-IR neurons nor for the quantification of the AVP-mRNA the raters were blind to the ante-mortem diagnosis, but the measurements were standardized such that this could not have influenced the study outcome.

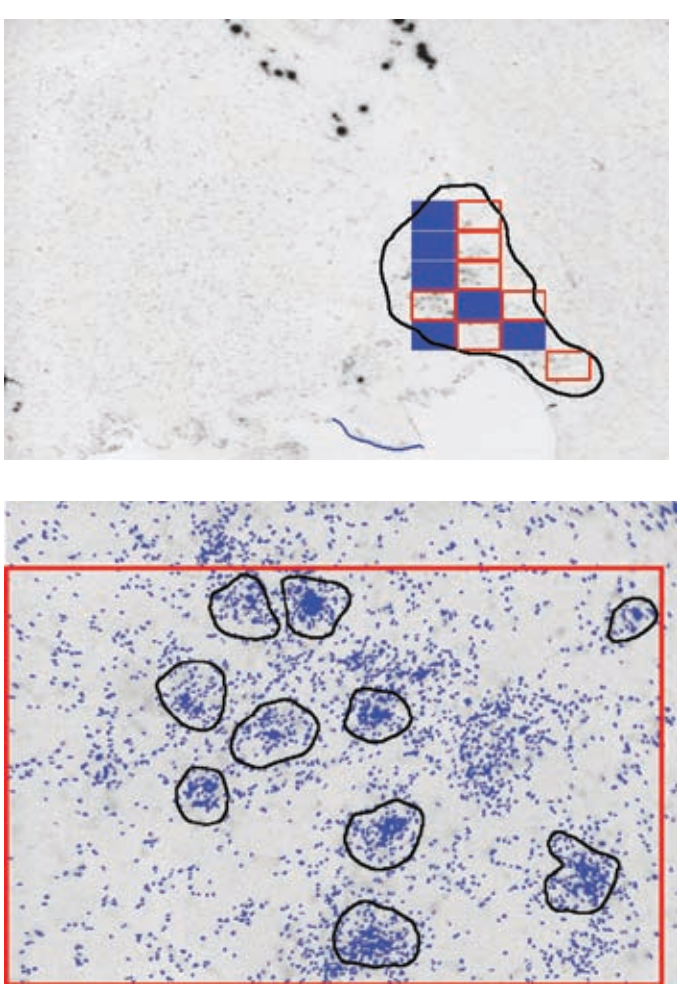


Figure 1
A: outline of the SCN at low magnification (2.5x objective) at the IBAS monitor with superimposed grid and selected fields; B: outline of positive profiles at high magnification (40x objective) at the IBAS monitor with superimposed mask over the silvergrains and red inclusion line

Statistical analysis

Differences among the groups were statistically evaluated by the Wilcoxon Signed Ranks Test (two-tailed). P values less than 0.05 were considered to be statistically significant. All values are expressed as the mean ± the standard deviation (SD). Differences within the depressed group according to their medication in the last month were tested with the non-parametric Mann-Whitney U test.

Linear regression analysis was performed to study the effects of postmortem delay and the duration of the disease on the AVP data set, using Spearman's correlation coefficient.

Results

The groups were matched for sex, age and clock time of death . Both groups consist of 4 female and 7 male subjects. Data on age (67± 8.7 for depressed subjects; 70 ± 12 for control subjects), brain weight (1280 g± 162.3 for depressed subjects; 1399 g ± 307.5 for control subjects; P=0.96), clock time of death, postmortem delay time (PMD) and fixation time (38 h± 12.5 for depressed subjects; 34.4 h ± 15.5 for control subjects; P=0.48) are presented in Table I. There were no differences in these factors between the control and depression group except for

the PMD. The control group had a shorter average PMD (9.5 ± 4.7h) than the depression group (32.5 ± 20.4 h) (Z=-2.67; P=0.008), but no significant relationship was found between PMD and the number of AVP-IR neurons (r_s=-0.200, P=0.56 for the depressed subjects; r_s=0.336, P=0.312 for the controls) or amount of AVP-mRNA (r=0.150, P=0.66 for the depressed subjects; r_s=0.203, P=0.55 for the controls).

The number of AVP-IR neurons in depression (6,589±2389) was found to be significantly higher than in controls (3,706±1678) (Z=-2.40; P=0.016) (Fig. 2). A clearly smaller amount of AVP-mRNA was found in the SCN of the subjects with depression (Fig.2). In depressed subjects, the total mask area of silver grains, as an estimate of total amount of AVP-mRNA in the SCN, was approximately half that of control subjects (5,921 ± 3,802 μm^2 vs 12,206 ± 5,827 μm^2) (Z=-2.49; P=0.013). Furthermore, the mean area of masked silver grains per profile was significantly lower in depressed subjects (0.33 μm^2± 0.11) compared to control subjects (0.52 μm^2 ± 0.15)(Z=-2.85; P=0.004). Although there was a tendency towards a lower number of profiles that expressed AVP-mRNA in the SCN in the depressed subjects (16,072 ± 8036) than in the controls (23,372 ± 8202), this difference did not reach significance (Z=-1.87; P=0.06).

There was no difference in the number of AVP-IR neurons (Z=-0.57; P=0.65) nor in the amount of AVP-mRNA (Z=-0.95; P=0.41) between four subjects who had taken lithium in the past (D1, D4, D7, D11; D1 and D7 took lithium in the last month prior to death) and the other depressed subjects. In addition, we did not find any difference in the number of AVP-IR neurons (Z=-0.38; P=0.79) or AVP- mRNA (Z=-0.57; P=0.65) between the subjects who took benzodiazepines (D4, D5, D7, D8, D9, D10, D11) during the last month before death and the other subjects. The number of AVP-IR neurons and AVP-mRNA in three subjects who were treated with selective 5-HT re-uptake inhibitors during the last month before death (D2 with fluoxetine, D5 with fluvoxamine and D10 with paroxetine) did not differ from the other subjects (Z=-0.612; P=0.63 and Z=-0.41; P=0.78 respectively).

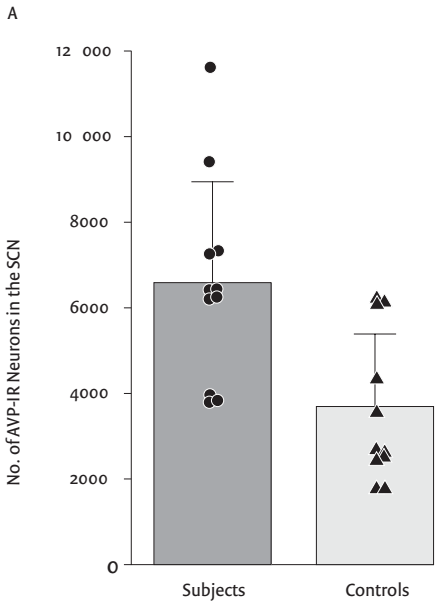
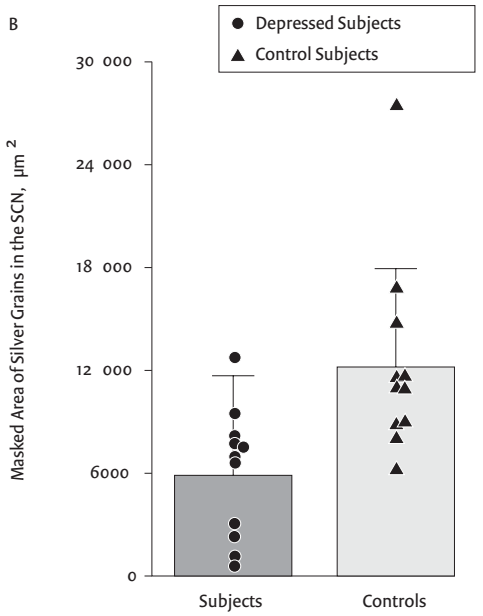


Figure 2.
The number of vasopressin-immunoreactive (AVP-IR) neurons (left two columns) and the mask area of silver grains of the AVP-mRNA (right two columns) in the suprachiasmatic nucleus (SCN) in control subjects (n=11) and depressed subjects (n=11). The error bars indicate the SD. Note the change in the balance between the presence of more AVP and less AVP-mRNA in depression.

There was no relationship between the number of AVP-IR neurons and the duration of the disease (from less than one year to 51 years) (r_s=.045; P=0.89) nor for the amount of AVP-mRNA and the duration of the disease (r_s=0.009; P=0.98).

The differences in the number of AVP-IR neurons and the amount of AVP-mRNA between depressed subjects and matched controls did not change in significance when the bipolar disordered subjects were left out from the analysis (Z=-2.52; P=0.012 and Z=-2.10; P=0.036 respectively).



Comment

In the present study, we found that the number of AVP-IR neurons in the SCN was higher in depressed subjects than in control subjects. At the same time, the expression of AVP-mRNA in the SCN was lower in depressed subjects compared to control subjects. The difference in AVP-mRNA is at least partly due to a decrease in the mean AVP production per neuron. These findings indicate a change in the balance between the production and transport of AVP in depression. A functional alteration of neurons in the SCN is in line with circadian rhythm disturbances which have been found in depression, e.g., in sleep-wakefulness, body temperature, hormonal rhythms and the periodicity of manic-depressive cycles in some bipolar disordered patients[3, 4].

As mentioned in the introduction we hypothesized that the number of AVP-IR neurons in the SCN would be decreased. This would be in line with an attenuated inhibition of AVP from the SCN on CRH neurons in the PVN[14], which could explain the increased number of CRH neurons together with increased CRH-mRNA levels in the PVN[10, 11]. Finally, this would lead to the frequently found increased levels of cortisol in depression. It was thus a surprise to find just the opposite, namely an increased number of AVP-IR neurons in the SCN in depression. We then wanted to know whether this increase was also reflected by the production of AVP in these neurons and performed an in situ hybridization for AVP-mRNA. The results of this experiment brought us back to our hypothesis, because we found a clearly decreased amount of AVP-mRNA in depression. Probably there is accumulation of AVP in the neurons of the SCN in depression due to a decreased transport rate of the neuropeptide. AVP is normally transported from the SCN to it's target areas by axonal transport. So far there is not much known about changes in transport rate related to psychiatric diseases, but in Alzheimer's disease a decreased axonal transport rate of the neurotrophin/trk complex due to cytoskeletal changes may be the underlying event for the neuronal atrophy in the nucleus basalis of Meynert[26]. The possibility of a decreased axonal transport rate in depression certainly needs further investigation.

It should be mentioned that the number of cell-profiles that expressed AVP-mRNA was higher than the total number of AVP peptide expressing neurons (controls in this study). This is due to the fact that in the ISH-study, profiles of cells were counted instead of the number of cells as estimated in the immunocytochemical study. For a comprehensive discussion on the use of the deconvolution or unfolding technique, we refer to a previous study at our institute by Raadsheer et al. (1994)[27]. In this paper a comparison is made between the use of the unfolding method and the disector and a high correlation was found between both methods ($r_s = 0.981$).

Information on the exact influence of antidepressants on the SCN, and more specifically AVP in the SCN, is limited. Lithium acts on hamster SCN neuronal firing in vitro, although it is not known on what type of neurons[28]. It has also been shown that the diurnal rhythm of AVP-mRNA in the rat SCN did not seem to

be affected by benzodiazepines[29]. Depletion of serotonin (5-HT) in the SCN has been shown to disrupt phase and period characteristics of the daily locomotor rhythm in rat and hamster[30, 31]. However, the diurnal rhythm of AVP-mRNA of the rat SCN in tissue culture was not disrupted following the administration of the 5-HT depleting agent parachlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor[31]. All these observations argue against treatment effects and support the idea that the alteration of AVP neurons in the SCN might well be related to the trait of depression per se. Our sample was however too small to draw any firm conclusions on the effect of treatment on the outcome measures.

With respect to a possible confounding effect of alcohol on AVP neurons in the SCN in humans nothing is known. One study by Harding[32] described that the use of high doses of alcohol is correlated with neuronal degeneration of magnocellular vasopressin neurons in the PVN and SON. They did, however, not describe an effect on the parvicellular vasopressin neurons in the SCN. In the rat SCN, Madeira[33] studied the effect of ethanol-treatment and withdrawal on AVP-immunoreactivity and mRNA levels in the rat SCN. They found a reduction in the number of AVP neurons in the SCN in both the ethanol treated and withdrawn rats. Also the hybridization signal for AVP-mRNA was reduced in both the ethanol treated and withdrawn rats, with even a weaker signal in the withdrawn rats. This makes it clear that not only the use of alcohol at the time of death should be taken into consideration, but also a possible irreversible effect after alcohol withdrawal during life time that could still confound the immuno-cytochemistry and in situ hybridization findings. However, none of the subjects used alcohol at the time of death, as reported by the medical scores. Only two subjects, D3 and C3, have a history of alcohol abuse, but these subjects were matched with each other and did not influence our conclusions.

Since the SCN is the clock of the brain, the time of death should also be considered as a possible confounding factor. We excluded this possibility by matching depressed subjects as much as possible with control subjects who had died around the same time (Table I). Moreover, a higher number of AVP-IR neurons and a lower amount of AVP-mRNA were found in depressed subjects over the entire period of the day and night (Fig. 3).

The functional alterations of AVP neurons in the SCN of depressed subjects are of special interest in relation to the impaired regulation of the HPA system in depressio[34]. Animal data show that AVP neurons of the SCN exert an inhibitory influence upon CRH in the PVN and thereby reduce the stress-induced release of glucocorticoids[14]. Increased levels of circulating glucocorticoids increase AVP-mRNA in the SCN within a narrow time window[35], which will strengthen the inhibition of CRH in the PVN. How exactly the SCN and the HPA-axis are linked to the pathobiology of depression needs further investigation on, e.g., the feedback mechanism of glucorticoids on the HPA-axis and on how the SCN is involved in this feedback.

Since this study was performed on postmortem human brain material, ante- and postmortem factors such as agonal state, medication, postmortem delay (PMD), duration of fixation and storage time of the tissue may contribute to the variation observed in mRNA levels[36, 37]. Information on the exact influence of each of these factors on AVP-mRNA levels, however, is still very limited. As far as PMD is concerned, a significant decrease in the amount of AVP-mRNA with increasing PMD was indeed shown in postmortem rat brain[36, 38, 39]. Relatively few ISH studies on postmortem effects on human brain material have been performed. Using ISH, several human mRNAs have been localized after a PMD of up to 40 h[23, 40]. In addition, no significant correlation was found between the density of the hybridization signal and PMD (range: 2.5-66 h) in a comparison of pro-opiomelanocortin mRNA levels in pituitaries between controls and different diseased patients[41]. Lucassen et al. reported that after 6 hours no further decrease in signal was detected in the AVP-mRNA in the

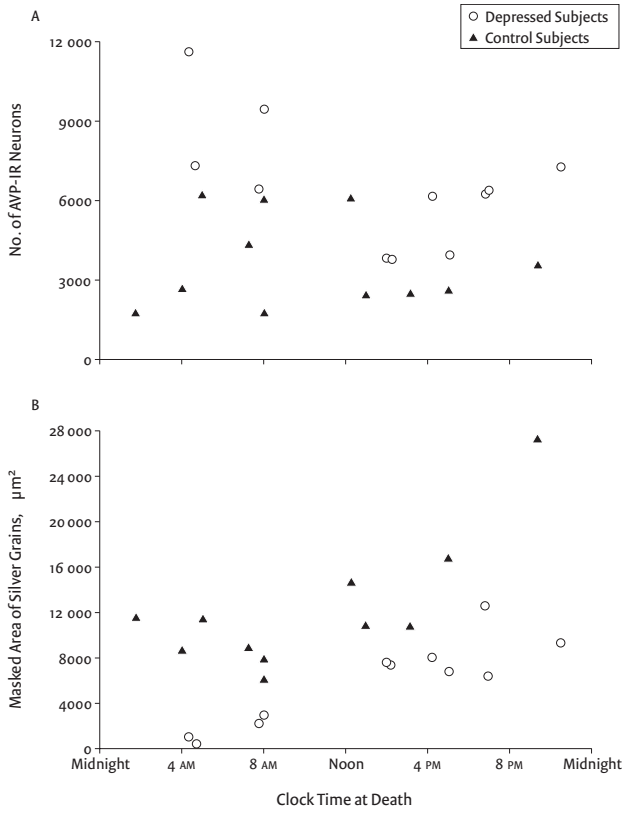


Figure 3.
A: Number of AVP-IR neurons plotted against clock-time of death of each individual (depressed subjects ▲ n =11; control subjects ▲ n = 11)
B: Area of masked silver grain plotted against clock-time of death of each individual (depressed subjects, n =11; control subjects ▲ n = 11)
This figure illustrates that the difference between depressed and control subjects is present at different timepoints of the day and that there is no overlap between the two groups when you take the clocktime of death into account.

human supraoptic nucleus and paraventricular nucleus of the hypothalamus. In our material the postmortem delay was 6 hours or longer (Table I). We did not find a significant correlation between the number of AVP-IR neurons and PMD nor between the amount of AVP-mRNA and PMD in the present study in either the control or in the depressed group, so that there is no indication that PMD might have influenced our conclusions.

To conclude, we found an alteration of the AVP neurons in the SCN in depressed subjects, both at the level of AVP-peptide and AVP-gene expression. The results suggest that the functional ability of the SCN to maintain normal biological rhythms is diminished in subjects suffering from depression, which seems to be the result of changes in the balance between production and transport of AVP in the SCN.

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References

1. Meijer, J. H. & Rietveld, W. J. Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. Physiol Rev 69, 671-707 (1989).

2. Swaab, D. F., Van Someren, E. J., Zhou, J. N. & Hofman, M. A. Biological rhythms in the human life cycle and their relationship to functional changes in the suprachiasmatic nucleus. Prog Brain Res 111, 349-68 (1996).

3. Souetre, E. et al. Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality. Psychiatry Res 28, 263-78 (1989).

4. Van den Hoofdakker, R. H. Chronobiological theories of nonseasonal affective disorders and their implications for treatment. J Biol Rhythms 9, 157-83 (1994).

5. Wirz-Justice, A. in Psychopharmacology: The Fourth Generation of Progress (eds. Bloom, F. E. & Kupfer, D. J.) 999-1017 (Raven Press, New York, 1995).

6. Eastman, C. I., Young, M. A., Fogg, L. F., Liu, L. & Meaden, P. M. Bright light treatment of winter depression: a placebo-controlled trial. Arch Gen Psychiatry 55, 883-9 (1998).

7. Rosenthal, N. E. et al. Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry 41, 72-80 (1984).

8. Yamada, N., Martin-Iverson, M. T., Daimon, K., Tsujimoto, T. & Takahashi, S. Clinical and chronobiological effects of light therapy on nonseasonal affective disorders. Biol Psychiatry 37, 866-73 (1995).

9. Moore, R. Y. The fourth C.U. Ariens Kappers lecture. The organization of the human circadian timing system. Prog Brain Res 93, 99-115 (1992).

10. Raadsheer, F. C., Hoogendijk, W. J., Stam, F. C., Tilders, F. J. & Swaab, D. F. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology 60, 436-44 (1994).

11. Raadsheer, F. C. et al. Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. Am J Psychiatry 152, 1372-6 (1995).

12. Holsboer, F., Spengler, D. & Heuser, I. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. Prog Brain Res 93, 385-417 (1992).

13. Stenzel-Poore, M. P., Heinrichs, S. C., Rivest, S., Koob, G. F. & Vale, W. W. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. J Neurosci 14, 2579-84 (1994).

14. Kalsbeek, A., Buijs, R. M., van Heerikhuize, J. J., Arts, M. & van der Woude, T. P. Vasopressin-containing neurons of the suprachiasmatic nuclei inhibit corticosterone release. Brain Res 580, 62-7 (1992).

15. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. (American Psychiatric Press, Washington, DC, 1994).

16. Hofman, M. A. & Swaab, D. F. Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. Brain Res 651, 134-42 (1994).

17. Swaab, D. F., Fliers, E. & Partiman, T. S. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res 342, 37-44 (1985).

18. Zhou, J. N., Hofman, M. A. & Swaab, D. F. Morphometric analysis of vasopressin and vasoactive intestinal polypeptide neurons in the human suprachiasmatic nucleus: influence of microwave treatment. Brain Res 742, 334-8 (1996).

19. Weibel, E. R. in Practical Methods for Biological Morphometry (ed. Weibel, E. R.) 186-196 (Academic Press Inc., London, England, 1979).

20. Cruz-Orive, L. M. Particle shape-size ditributions - the general spheroid problem, II: stochastic model and practical guide. J Microsc 112, 153-167 (1978).

21. Gundersen, H. J. Notes on the estimation of the numerical density of arbitrary particles: the edge effect. J Microsc 111, 219-223 (1977).

22. Mohr, E., Hillers, M., Ivell, R., Haulica, I. D. & Richter, D. Expression of the vasopressin and oxytocin genes in human hypothalami. FEBS Lett 193, 12-6 (1985).

23. Mengod, G., Charli, J. L. & Palacios, J. M. The use of in situ hybridization histochemistry for the study of neuropeptide gene expression in the human brain. Cell Mol Neurobiol 10, 113-26 (1990).

24. Mengod, G., Goudsmit, E., Probst, A. & Palacios, J. M. In situ hybridization histochemistry in the human hypothalamus. Prog Brain Res 93, 45-55 (1992).

25. Lucassen, P. J. et al. In situ hybridization for vasopressin mRNA in the human supraoptic and paraventricular nucleus; quantitative aspects of formalin-fixed paraffin-embedded tissue sections as compared to cryostat sections. J Neurosci Methods 57, 221-30 (1995).

26. Salehi, A. et al. Decreased neuronal activity in the nucleus basalis of Meynert in Alzheimer's disease as suggested by the size of the Golgi apparatus. Neuroscience 59, 871-80 (1994).

27. Salehi, A., Verhaagen, J., Dijkhuizen, P. A. & Swaab, D. F. Co-localization of high-affinity neurotrophin receptors in nucleus basalis of Meynert neurons and their differential reduction in Alzheimer's disease. Neuroscience 75, 373-87 (1996).

28. Raadsheer, F. C., Oorschot, D. E., Verwer, R. W., Tilders, F. J. & Swaab, D. F. Age-related increase in the total number of corticotropin-releasing hormone neurons in the human paraventricular nucleus in controls and Alzheimer's disease: comparison of the disector with an unfolding method. J Comp Neurol 339, 447-57 (1994).

29. Mason, R. & Biello, S. M. A neurophysiological study of a lithium-sensitive phosphoinositide system in the hamster suprachiasmatic (SCN) biological clock in vitro. Neurosci Lett 144, 135-8 (1992).

30. Carter, D. A. & Murphy, D. Diurnal rhythm of vasopressin mRNA species in the rat suprachiasmatic nucleus: independence of neuroendocrine modulation and maintenance in explant culture. Brain Res Mol Brain Res 6, 233-9 (1989).

31. Morin, L. P. & Blanchard, J. Depletion of brain serotonin by 5,7-DHT modifies hamster circadian rhythm response to light. Brain Research 566, 173-85 (1991).

32. Harding, A. J., Halliday, G. M., Ng, J. L., Harper, C. G. & Kril, J. J. Loss of vasopressin-immunoreactive neurons in alcoholics is dose- related and time-dependent. Neuroscience 72, 699-708 (1996).

33. Madeira, M. D. et al. Chronic alcohol consumption and withdrawal do not induce cell death in the suprachiasmatic nucleus, but lead to irreversible depression of peptide immunoreactivity and mRNA levels. J Neurosci 17, 1302-19 (1997).

34. Deuschle, M. et al. Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. J Clin Endocrinol Metab 82, 234-8 (1997).

35. Larsen, P. J. et al. The diurnal expression of genes encoding vasopressin and vasoactive intestinal peptide within the rat suprachiasmatic nucleus is influenced by circulating glucocorticoids. Brain Res Mol Brain Res 27, 342-6 (1994).

36. Barton, A. J., Pearson, R. C., Najlerahim, A. & Harrison, P. J. Pre- and postmortem influences on brain RNA. J Neurochem 61, 1-11 (1993).

37. Burke, W. J. et al. Effect of pre- and postmortem variables on specific mRNA levels in human brain. Brain Res Mol Brain Res 11, 37-41 (1991).

38. Arai, H., Noguchi, I., Sagi, N., Moroji, T. & Iizuka, R. A study of non-isotopic in situ hybridization histochemistry on postmortem changes in vasopressin mRNA in rat brain. Neurosci Lett 103, 127-32 (1989).

39. Noguchi, I., Arai, H. & Iizuka, R. A study on postmortem stability of vasopressin messenger RNA in rat brain compared with those in total RNA and ribosomal RNA. J Neural Transm Gen Sect 83, 171-8 (1991).

40. Sola, C., Mengod, G., Probst, A. & Palacios, J. M. Differential regional and cellular distribution of beta-amyloid precursor protein messenger RNAs containing and lacking the Kunitz protease inhibitor domain in the brain of human, rat and mouse. Neuroscience 53, 267-95 (1993).

41. Mengod, G., Vivanco, M. M., Christnacher, A., Probst, A. & Palacios, J. M. Study of pro-opiomelanocortin mRNA expression in human postmortem pituitaries. Brain Res Mol Brain Res 10, 129-37 (1991).

Chapter 5

Comparison between informant observed and actigraphic assessment of sleep-wake rhythm disturbances in demented residents of homes for the elderly

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Abstract

Objective: Sleep-wake rhythm disturbances frequently occur in demented elderly and are of clinical relevance since they herald accelerated functional decline and institutionalization. Assessment of sleep-wake rhythm disorders is therefore of significant importance, and can be performed by questionnaires or actigraphy, i.e. the recording of wrist activity. The present study investigates the relation of these two types of measurement, by simultaneously assessing actigraphy and the Circadian Sleep Inventory for Normal and Pathological States (CSINAPS).
Method: 78 elderly subjects, mean age 85.2 ± 5.8 years, living in group care facilities of twelve homes for the elderly, wore an actigraph during two weeks. Caregivers completed the nurse informant CSINAPS. Spearman rank correlations and Mann-Whitney U tests were calculated over the equivalent sleep-wake rhythm parameters as derived from actigraphy and from the CSINAPS.
Results: Good correlations were found between questionnaire items about habitual timing of sleep and wakefulness and their actigraphic counterparts. Caregivers overestimated the actual sleep time between sleep onset and offset by 96 minutes. Questionnaire reports of sleep disturbances like wandering at night were also reflected in actigraphy parameters. However, the questionnaire and actigraphy variables correlate only modestly and may complement each other. In our study, both actigraphy and the CSINAPS seemed to miss the previously established high prevalence of sleep disordered breathing (SDB) and leg movements during sleep (LM).
Conclusion: The assessment of sleep and wake disturbances in demented elderly is best served by parallel use of a questionnaire like the CSINAPS and actigraphy. Moreover, if SDB and LM are a focus of interest, additional assessments are needed.

Introduction

Sleep-wake rhythm disturbances frequently occur in Alzheimer's disease (AD). The disturbances include increased wakefulness and wandering at night, daytime napping, and an instable and fragmented sleep-wake rhythm with a reduced 24-hour amplitude [1-6]. Functional changes in the circadian timing system, especially in the endogenous biological clock located in the hypothalamic suprachiasmatic nucleus (SCN), are thought to play a key role in the 24-hour rhythm disturbances [7, 8]. Sleep-wake rhythm disturbances predict accelerated functional decline and institutionalization [5, 9, 10] and have a strong negative impact on caregiver burden. Monitoring sleep-wake rhythm disturbances is of importance for clinical care as they may be ameliorated by chronobiological or behavioral treatment [8, 11]. Unobtrusive monitoring can be done either through observation using informant questionnaires or by actigraphy, the long-term continuous assessment of wrist activity. The aim of the present study was to investigate the cross-validity, strengths and weaknesses of these methods.

Caregivers may provide accurate information about the patient [12]. Kaufer et al. [13] proposed a questionnaire specifically tailored to the sleep-wake rhythm disturbances in dementia. The Circadian Sleep Inventory for Normal and Pathological States (CSINAPS) includes items about sleep habits and disturbances. The CSINAPS was able to distinguish normal elderly from AD patients regarding the presence of circadian and sleep disturbances. We here present cross-validity of the CSINAPS scores with objectively measured sleep-wake rhythm parameters in demented elderly in a nursing home setting.

The golden standard for objective assessment of disturbed sleep, polysomnography, is deemed to fail in many demented subjects while actigraphy is tolerated by the majority. Actigraphy allows for objective estimation of sleep and sleep-wake rhythms [14]. Actigraphic estimates have been found to reliably reflect sleep/wake cycles [14-17]. Reasonable conver-

gence of simultaneously assessed actigraphic and polysomnographic sleep estimates have been demonstrated in elderly subjects [18] insomniacs [19] and demented patients [14, 20-22]. The present study compared simultaneously assessed indirect observations (CSINAPS) and actigraphy as two methods to obtain sleep-wake rhythm disturbances in a group of mainly demented elderly.

Methods

Subjects

Elderly (n=78; 73 female) were recruited from group care facilities of 12 homes for the elderly. Mean age was 85 ± 6 years (range 70-97). The average Mini Mental State Examination (MMSE) was 14.8 ± 6.8 [23]. The clinical diagnosis of dementia was made according to the DSM-IV criteria for dementia and dementia subtypes [24] and the NINCDS-ADRDA criteria for probable or possible Alzheimer's Disease [25]. Subjects were diagnosed with probable Alzheimer's disease (n = 52), vascular dementia (n = 8), other types of dementia (n=12) or turned out not to be demented (n = 6). The study was approved by the Medical Ethics Committee of the Gelderse Vallei Hospital, Ede, The Netherlands, and relatives signed informed consent.

Materials

Circadian sleep inventory for normal and pathological states

The CSINAPS [13] consists of two parallel forms, the self-rated form ("S-" form) and the informant-rated form for cognitive-impaired geriatric patients who are unable to answer the questions themselves ("I-" form). The latter was administered to the professional caregivers of the patients, and the night duty nurse answered the questions about nocturnal behavior. The CSINAPS asked for a retrospective of the past weeks, during which the actigraph was worn. The CSINAPS allows symptoms to be rated in terms of their frequency, severity and impact. The latter was not included in the present study since this should be filled out by a spouse living with the patient. The questionnaire includes (1) sleep habits, (2) sleep-wake disturbances and (3) circadian behavioral disturbances. The original questionnaire can be requested from DK at kauferd@neurology.unc.edu. Items on sleep habits ask for *Bed Time* and *Get Up Time*, *Total Sleep Time*, *Nap frequency*, *Nap duration*, and *Nap Periods*. Ten items investigate sleep- wake disturbances, and were proposed to be aggregated in the three subscales *Sleep Initiation and Continuity*, *Day- Night Alterations* and *Respiratory/Motor/Dreaming* [13]. The items on *Circadian Behavioral Disturbances* include wandering, agitation, confusion and combativeness. Confirmative answers on the sleep-wake disturbances and circadian behavioral disturbances are followed-up by 4-point ratings for both frequency and severity. Items are scored as 0 for 'No' and the multiplication of frequency and severity for present symptoms. Since both frequency and severity can be scored with a 1 to 4, this multiplication can range from 1 to 16. The subscales sum their included items and in case of missing items an estimate was derived by multiplying the average of the present items by the original number of items belonging to the scale.

Actigraphy

Subjects wore an Actiwatch (Cambridge Neurotechnology Ltd, Cambridge, UK) on the non-dominant wrist continuously for two weeks. Unintended removal of the actiwatch by the subjects was to a large extent prevented by using a home-made key-ring locking system. Periods of prolonged zero activity, suggesting that the actiwatch was, in spite of this adjustment, not worn for several hours to days have not been included in the analysis. Average adequate recording duration per subject was 12.9 ± 3.5 days and nights.

Estimates of sleep parameters were obtained using the validated [19]. Sleepwatch Analysis Software 2001 (Cambridge Neurotechnology Ltd., Cambridge, UK) with high sensitivity settings [18]. In order to differentiate between CSINAPS and actigraphy variables, the first appear in *italics* and the latter with

capital first letters throughout this paper. The software automatically calculates Sleep Start and Sleep End, which are limited to occur at any time between the habitual *Bed Time* and *Get Up Time* as read from the caregivers reports and entered into the software; Assumed Sleep, i.e. the difference between Sleep End and Sleep Start, Actual Sleep Time, the amount of sleep as determined by the algorithm and is equivalent to Assumed Sleep minus wake time. We defined Sleep Efficiency as the percentage of actual sleep time between sleep onset and final awakening, i.e. excluding sleep onset latency, of which the estimate is poor in the absence of day-by-day precise lights-off times. Mean Activity Score is the average activity score in those epochs where scores of greater than zero were recorded during the assumed sleep period.

Nonparametric variables quantified the circadian rest-activity pattern as previously described [20, 22]. In brief, the Interdaily Stability (IS) gives an indication of the stability of the 24-hour rest-activity pattern over days and varies from 0 to 1, where 1 means a high stability. The Intradaily Variability (IV) gives an indication of the fragmentation of the rhythm, by quantifying the number and strength of transitions between periods of rest and of activity. IV increases with more fragmented sleep-wake patterns. L5 quantifies the activity level during the core sleep period. High L5 values indicate nocturnal restlessness. L5- onset phase indicates the start-time of this most restful 5-hour period. M10 describes the daytime activity level. Low M10 values indicate that the subject is inactive during the day. M10- onset phase indicates the start-time of this most active 10-hour period. AMP is an absolute amplitude measure and calculated as the difference between M10 and L5. RA is the relative amplitude, calculated by dividing AMP by the sum of L5 and M10.

Statistical analysis

The data were analyzed using the SPSS (Chicago, IL). Spearman's rho correlations and Mann-Whitney U tests were calculated to compare the CSINAPS variables with analogous sleep-wake and circadian variables derived from the actigraphic data. One-tailed tests of significance were calculated with p-values < 0.05 being considered as significant, since all hypotheses on relations were clearly unidirectional. Cronbach's alphas were calculated as measures of the internal consistency of the subscales and the complete list of items of the CSINAPS.

Monte Carlo simulations provided the following statistical power estimates [26]. For the Mann-Whitney U tests, one tailed testing at $p=0.05$ requires two samples of 31 cases to have a statistical power of 0.80 to detect a medium to large effect (0.65, following the convention of 0.5 for a medium sized effect and 0.8 for a large effect). For the Spearman correlations, one tailed testing at $p=0.05$ requires a sample of 42 cases to have a statistical power of 0.80 to detect a medium to large correlation (0.40, following the convention of 0.3 for a medium sized correlation and 0.5 for a strong correlation).

Results

CSINAPS item and subscale averages are summarized in table 1. None of the items was completed for all 78 subjects, indicating that the caregivers could not answer all questions. The items about snoring and combative or violent behavior during the night were not answered confirmatively in any of the subjects. Table 1 shows the averages of all actigraphy parameters used for comparison with the CSINAPS scores.

Sleep habit items

CSINAPS variables are presented in *italics* and actigraphic parameters with capital first letters. Sleep habits according to the CSINAPS (single item level) and according to actigraphy were significantly correlated between *Bed Time* and Sleep Start (rho= 0.88, n=73, $p< 0.005$), and between *Get up time* and Sleep End (rho= 0.92, n=62, $p< 0.005$). These correlations were not surprising, since the

actigraphy software calculation of Sleep Start and Sleep End is in part based on questionnaire data on the habitual *Bed Time* and *Get Up Time*. *Estimated Total Sleep Time* and Assumed Sleep Time correlated strongly (rho= 0.48, n=55, p< 0.005). The *Estimated Total Sleep Time* was only moderately correlated with actigraphic Actual Sleep Time (rho = 0.25, n = 55, p< 0.04). Together, these findings suggest that caregivers may give less than optimal estimates of the time actually spent asleep between sleep onset and final awakening. The difference of caregiver estimated sleep time and actigraphy estimated actual sleep time was 96±128 minutes. Caregivers thus overestimate the actual time asleep by more than one and a half hour on average.

Subjects reported to nap during the day according to the CSINAPS (n=54) did not show a significantly lower actigraphic daytime activity level (M10) than subjects reported not to nap (n= 20) (M10 = 97.09 ± 69.94 and 102.3 ± 59.09, respectively, U= 468, p = 0.19).

Sleep and circadian disturbance items

For a comparison of actigraphic counterparts of the CSINAPS scores on questions (single item level) about sleep wake disturbances, subjects were grouped according to presence or absence of each evaluated CSINAPS disturbance item.

Subjects for whom caregivers reported *waking up* in the middle of the night did not show a significantly higher L5 (n= 17, L5 = 18.10 ± 11.72) than those who were reported *not to wake up* in the night (n= 36, L5 = 14.80 ± 12.29) (U= 241, p= 0.11). On the other hand, the Mean Activity Score was higher in the former (94.47 ± 276.76) as compared to the latter group (19.33 ± 13.87) (U = 206, p < 0.03). This finding indicates the integrated activity score over the whole night (Mean Activity Score) to be more sensitive to nocturnal wakefulness than the integrated activity score over the core five hours of sleep (L5).

Subjects reported to show *daytime fatigue* had a lower Interdaily Stability (n= 20, IS = 0.52 ± 0.17) than the group of subjects without *daytime fatigue* (n= 50, IS=0.43 ± 0.15) (U = 570, p < 0.04).

Subjects for whom caregivers reported *increased daytime* sleep had a 36 % lower Daytime Activity Level (n= 11, M10 = 69.54 ± 35.69) than those who were reported not to show *increased daytime sleep* (n= 57, M10 = 108.69 ± 75.58) (U = 211, p < 0.05). They also had a 23% lower Interdaily Stability (n= 11, IS = 0. 40 ± 0.18 versus n= 57, IS = 0.52 ± 0.17) (U = 184, p < 0.02), and a 14% higher Intradaily Variability (n= 11, IV = 1.48 ± 0.22 versus n= 57, IV = 1.29 ± 0.28) (U = 172, p < 0.01).

Subjects who *wandered at night* showed a 17% lower Sleep Efficiency (n=8, 66.38 ± 18.41) than the subjects who did *not wander at night* (n = 67, 79.95 ± 10.29) (U = 141, p < 0 .02), and a 247 % higher L5 (52.43 ± 36.15 versus 15.10 ± 11.47) (U = 75, p < 0.0005).

CSINAPS subscales

The internal consistencies of the CSINAPS subscales as indicated by Cronbach's alpha were as follows. Note that there are different sample sizes for the different subscales due to items that had not been filled out by the caregivers: they represent the number of completely filled out forms for that subscale: *Sleep Initiation and Continuity* (n=38), alpha = 0.66; *Day- Night Alterations* (n=56), alpha = 0.72; *Respiratory/ Motor/ Dreaming* (n=42, if excluding the item about snoring), alpha = 0.12; Circadian Behavioral Disturbances (n=67, if excluding the item about violent behavior) alpha =0 .57; CSINAPS Total (n=19) alpha = 0.77.

The Cronbach's alpha of subscale *Respiratory/ Motor/ Dreaming* is very low in the present sample. Many missing values were present in the four items covering this scale, and confirmative answers hardly occurred for the items about snoring (0 times), breathing problems (1 time), unusual movements (1 time) and disturbing dreams (8 times).

Spearman's rho rank correlations were

Table 1.

			Sleep parameters				
			Actigraphy variable	Sleep Start, hr	Sleep End, hr	Assumed Sleep Time, min	Actual Sleep Time, min
CSINAPS Items	N	Percentage Affirmative	Mean ± SD	21:54 ± 1:12	7:57 ± 0:52	602 ± 91	476 ± 108
Part I Sleep Habits				0.88			
Bed Time, hr	73		21:15±1:07		0.92		
Get up Time, hr	62		8:08±0:53			0.48	0.25
Total SleepTime, min	55		556±102				
Naps, yes/no	74	73%					
Part II Sleep-Wake Distrubances	78		9.25±13.19				
Sleep Initiation and Continuity	75		3.62±5.88		-0.20		
Sleep initiation	55	9%	0.78±2.82				
Sleep continuation	53	32%	1.75±3.38				
Early Awakening	59	15%	0.48±1.29				
Day/Night Alterations	77		3.68±8.05			0.19	
Tiredness/Fatigue	70	29%	2.13±4.24				
Daytime Sleep	68	16%	1.54±3.98				
Decreased Night Sleep	67	4%	0.246±1.51				
Respiratory/ Motor/Dreaming	72		1.63±7.12				
Snoring	43	0%	0±0				
Nocturnal respiratory difficulty	54	2%	0.019±0.14				
Abnormal Movements	46	2%	0.049±0.33				
Abnormal Dreams	68	12%	0.46±10.72				
Part III Circadian Behavioral Disturbances	77		0.64±0.92				
Wandering	75	11%	0.11±0.311				
Confusion	73	25%	0.25±0.434				
Agitation	70	27%	0.27±0.448				
Combative-Violent	70	0%	0±0				

Note: The left four columns, respectively, show for all CSINAPS times and their aggregated subscales: 1) the number of subjects for which the item/subscale could be obtained; 2) the percentage of affirmative answers for the specific problem asked for by the item; and 3) the sample mean ± standard deviation. The headers of the columns further to the right show the mean ± standard deviation for the actigraphic sleep parameters and circadian parameters.
The cross points of CSINAPS item/subscale rows and actigraphy variable columns show either, in case of comparisons between continuous variables the Spearman correlation (in italics) between the two, or in case of a dichotomous yes/no answer on the CSINAPS item the difference (in %) in actigraphic variable means for the group of subjects that do show the CSINAPS item problem versus those that do not. Only the significant results (one-sided alpha < 0.05) are shown. For example, the actigraphic variable L5 (nocturnal activity) correlates with the 10-item integrated Sleep-Wake Disturbances subscale (0.22), with the tree-item Sleep Initiation and Continuity subscale (0.30) and with the four-item Circadian Behavioral Disturbance Subscale (0.27). The latter appears mainly due to Nocturnal Wandering, which is reported to be present in eight out 75 subjects (11%). The mean L5 of these eight subjects is 247% higher as compared to the mean L5 of these subjects for which no Nocturnal Wandering has been reported (52.43 versus 15/10 arbitrary units).
SD: standard deviation; AMP: absolute amplitude measure; RA: relative amplitude.

Table 1. Continued

Sleep Parameters		Circadian Parameters							
Sleep Efficiency, %	Mean Activity Score	Interdaily Stability	Intradaily Variability	L5	L5-Onset Phase, hr	M10	M10-Onset Phase, hr	AMP	RA
78.6 ± 11.8	41 ± 131	0.50 ± 0.18	1.34 ± 0.30	19± 19	0:26 ± 2:27	102 ± 69	9:39 ± 3:05	83 ± 61	0.69 ± 0.18
-0.26	0.28			0.22				0.26	
-0.24	0.30		0.25	0.30	-0.23			-0.27	
	+389%								
		0.20							
		-17%							
		-23%	+14%			-36%			
-0.20	0.20	-0.20		0.27					
-17%				+247%					

calculated between the other subscales of the CSINAPS and their associated actigraphic sleep and circadian parameters. The CSINAPS subscale *Sleep Initiation and Continuity* was significantly correlated with three sleep parameters: Sleep End (rho = -0.20, n = 75, $p < 0.05$), Sleep Efficiency (rho = -0.24, n = 75, $p < 0.02$) and Mean Activity Score (rho = 0.30, $p < 0.005$). This subscale also correlated with four circadian parameters: Intradaily Variability (rho = 0.25, n=75, $p < 0.02$), L5 (rho = 0.30, n=75, $p < 0.005$), Relative Amplitude (rho= -0.27, n = 75, $p < 0.01$) and L5-onset-phase (rho= -0.23, n = 75, $p < 0.03$). The latter negative correlation indicates that problems with the continuity of sleep are worse in those subjects that have their major 5-hour sleep period early during the night.

The subscale *Day- Night Alterations* correlated with Assumed Sleep (rho = 0.19, n = 77, $p < 0.05$) and with the circadian parameter Interdaily Stability (rho = -0.20, n =77, $p < 0.05$). These findings indicate that observed changes in circadian patterning of sleep are

related to actigraphically measured longer times in bed (but not longer times asleep) and less predictable sleep-wake schedules.

The subscale *Circadian Behavioral Disturbances* correlated with Sleep Efficiency (rho = -0.20, n = 77, $p < 0.05$) and Mean Activity Score (rho = 0.20, n = 77, $p < 0.05$). This subscale also correlated with Interdaily Stability (rho=-0.20, n = 77, $p < 0.05$) and Nocturnal Activity L5 (rho= 0.27, $p < 0.01$).

When correlating the Total Score on all items of the CSINAPS measuring *Sleep-Wake Disturbances* (this sum score includes all items about sleep-wake disturbances, but not the *Circadian Behavioral Disturbances*) with the actigraphic data, we found significant correlations with Sleep Efficiency (rho = -0.26, n = 78, $p < 0.02$) and Mean Activity Score (rho = 0.28, n = 78, $p < .01$). We also found a negative correlation with L5, Nocturnal Activity (rho = 0.22, n = 78, $p < 0.03$) and the Relative Amplitude (rho= - 0.26, n = 78, $p<0.02$).

Discussion

There was good agreement between scores on the habitual sleep timing items and corresponding actigraphy parameters, as indicated by strong correlations between the CSINAPS items *Bed Time* and *Get Up Time* with the respective actigraphic variables Sleep Start and Sleep End. However, whereas caregivers adequately reported the timing of the major sleep period, they overestimated the amount actually slept by more than 30 minutes. Actigraphy may provide a more reliable estimate of actual sleep time.

At the CSINAPS single item level, *Waking Up In The Middle Of The Night* was better reflected in the whole night actigraphic parameter Mean Activity Score than in activity during the core five hours of sleep (L5). *Increased Daytime Sleep* was well reflected in M10, meant to quantify daytime activity, and in Interdaily Stability and Intradaily Variability, both meant to quantify circadian rhythm disturbances.

Nocturnal wandering was also well reflected by a lower Sleep Efficiency, and even more so in its related circadian variable L5, the average activity level in the five-hour period where activity is habitually minimal. Thus, whereas the actigraphic parameter Mean Activity Score may primarily reflect nocturnal awakening (see above), L5 seems to better reflect nocturnal wandering.

The higher sensitivity of the nonparametric variables IS, IV and L5 is in accordance with an earlier comparative study on the sensitivity of several actigraphic variables to circadian disturbances and treatment effects [22]. Contrary to expectation, naps reported by the nurses were not reflected in any of the actigraphic data, whereas it would be expected to be reflected at least in M10, and most likely also in IV. As mentioned above, M10, IV and IS *did* reflect the caregiver reported *Increased Daytime Sleep*. Together, these findings suggest that scheduled naps are complemented by periods of diurnal sleep occurring outside of these scheduled nap times, and that the more sensitive question to ask caregivers is about the occurrence of daytime sleep rather than the occurrence of naps.

The internal consistency in the subscales of the CSINAPS was good or satisfactory (Cronbach's alpha= 0.57 to 0.77) except for the subscale *Respiratory/ Motor/ Dreaming*. The lack of affirmative scores on the latter is remarkable, since a high prevalence of both sleep disordered breathing (SDB) and leg movements during sleep (LM) has convincingly been demonstrated in healthy and demented elderly [27, 28]. Our finding suggests that these disturbances most often go unnoticed by caregivers in homes for the elderly. Consequently, instruments other than actigraphy and questionnaires are needed to detect SDB and LM. Physiological assessments should be as unobtrusive as actigraphy given the poor compliance of demented elderly. Integration of assessment devices in the bedding would be most valuable. Otherwise, one should train caregivers on night-duty to focus on SDB and LM. The use of reports of bedpartners does not seem feasible since most

of the subjects are institutionalized single.

The subscale *Sleep Initiation and Continuity* was well reflected in actigraphic variables, including Sleep End, Sleep Efficiency, Mean Activity Score, Intradaily Variability, nocturnal activity L5 and relative amplitude RA. Problems with *Sleep Initiation and Continuity* also correlated negatively with the onset-phase of the main 5-hour sleep period L5. This negative correlation suggests that the problems are more related to the continuation of sleep in the early morning than to problems falling asleep in the early evening, which is in agreement with the general complaints of healthy elderly.

The subscale *Circadian Behavioral Disturbances* was represented in actigraphic variables Sleep Efficiency, Mean Activity Score, Interdaily Stability and nocturnal activity L5.

The findings suggest a reasonable cross validity of actigraphy and the CSINAPS questionnaire. The sizes of most correlations are between rho=0.20 and 0.30, which are according to convention better than small correlations (±0.10), but at best medium sized correlations (±0.30). Apparently, there is considerable residual variance, which supports the notion that the methods may best be used to complement each other. Furthermore, our findings suggest that the variables of the CSINAPS and of actigraphy reflect different aspects of the sleep-wake pattern, in clinical terms: more a syndrome than a (single) symptom. Ongoing validation studies suggest that the subscales should be seen as provisional, and subject to change (DK, unpublished).

Conclusion

The present paper compared the use of a questionnaire and actigraphy to quantify circadian and sleep disturbances in demented elderly. The CSINAPS is one of the few questionnaires available that is specifically designed to measure these disturbances in demented elderly (see also [29]). Our results should therefore be used with caution, and our study was primarily intended to evaluate the cross-validity.

To summarize, the main findings were as follows. First, caregivers should be given additional support in order to prevent missing data in filling out the questionnaire. Second, CSINAPS items asking for apnea and restless legs are seldom answered confirmative, whereas previous studies indicated a high risk in the demented population. Third, caregivers tend to overestimate the time actually spent asleep between sleep onset and offset. Fourth, correlations between the CSINAPS items and subscales and corresponding aspects of the actigraphic time are generally moderate at best, but all in the expected direction. We conclude that questionnaire and actigraphy data may complement each other, but that additional studies on the internal validity and factor structure of the CSINAPS would be useful.

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References

1. Cohen-Mansfield J, Billig N: Agitated behaviors in the elderly. I. A conceptual review, J Am Geriatr Soc 1986, 34:711-721

2. Cohen-Mansfield J, Marx MS, Rosenthal AS: A description of agitation in a nursing home, J Gerontol 1989, 44:M77-84

3. Cohen-Mansfield J, Werner P, Marx MS, et al.: Two studies of pacing in the nursing home, J Gerontol 1991, 46:M77-83

4. Martin J, Marler M, Shochat T, et al.: Circadian rhythms of agitation in institutionalized patients with Alzheimer's disease, Chronobiol Int 2000, 17:405-418

5. Moe KE, Vitiello MV, Larsen LH, et al.: Symposium: Cognitive processes and sleep disturbances: Sleep/wake patterns in Alzheimer's disease: relationships with cognition and function, J Sleep Res 1995, 4:15-20

6. van Someren EJ, Hagebeuk EE, Lijzenga C, et al.: Circadian rest-activity rhythm disturbances in Alzheimer's disease, Biol Psychiatry 1996, 40:259-270.

7. Swaab DF, Fliers E, Partiman TS: The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia, Brain Res 1985, 342:37-44

8. Van Someren EJ, Riemersma RF, Swaab DF: Functional plasticity of the circadian timing system in old age: light exposure, Prog Brain Res 2002, 138:205-231

9. Pollak CP, Perlick D: Sleep problems and institutionalization of the elderly, J Geriatr Psychiatry Neurol 1991, 4:204-210

10. Van Someren EJ: Circadian and sleep disturbances in the elderly, Exp Gerontol 2000, 35:1229-1237.

11. McCurry SM, Gibbons LE, Logsdon RG, et al.: Training caregivers to change the sleep hygiene practices of patients with dementia: the NITE-AD project, J Am Geriatr Soc 2003, 51:1455-1460

12. Ancoli-Israel S, Klauber MR, Gillin JC, et al.: Sleep in non-institutionalized Alzheimer's disease patients, Aging (Milano) 1994, 6:451-458

13. Kaufer D, Reynolds III C, Ketchel P, et al.: Circadian sleep disturbances in normal elderly, alzheimer´s patients, and their caregivers., Journal of the American Geriatrics Society 1998, 46:S11

14. Ancoli-Israel S, Clopton P, Klauber MR, et al.: Use of wrist activity for monitoring sleep/wake in demented nursing-home patients, Sleep 1997, 20:24-27

15. Ancoli-Israel S, Cole R, Alessi C, et al.: The role of actigraphy in the study of sleep and circadian rhythms, Sleep 2003, 26:342-392

16. Sadeh A, Hauri PJ, Kripke DF, et al.: The role of actigraphy in the evaluation of sleep disorders, Sleep 1995, 18:288-302

17. Sadeh A, Sharkey KM, Carskadon MA: Activity-based sleep-wake identification: an empirical test of methodological issues, Sleep 1994, 17:201-207

18. Colling E, Wright M, Lahr S, et al.: A Comparison of Wrist Actigraphy with Polysomnography as an Instrument of Sleep Detection in Elderly Persons, Sleep 2000, 23:a378

19. Kushida CA, Chang A, Gadkary C, et al.: Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients, Sleep Med 2001, 2:389-396

20. Van Someren EJ, Kessler A, Mirmiran M, et al.: Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients, Biol Psychiatry 1997, 41:955-963

21. Van Someren EJ, Lijzenga C, Mirmiran M, et al.: Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males, J Biol Rhythms 1997, 12:146-156

22. Van Someren EJ, Swaab DF, Colenda CC, et al.: Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer

patients by application of nonparametric methods, Chronobiol Int 1999, 16:505-518

23. Folstein MF, Folstein SE, McHugh PR: "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician, J Psychiatr Res 1975, 12:189-198

24. American Psychiatric Association: DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. Edited by Washington, DC, American Psychiatric Press, 1994, p

25. McKhann G, Drachman D, Folstein M, et al.: Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease, Neurology 1984, 34:939-944.

26. Brooks GP: Using Monte Carlo methods to teach statistics: The MC2G computer program., Understanding statistics 2003, 2:137-150

27. Ancoli-Israel S, Klauber MR, Butters N, et al.: Dementia in institutionalized elderly: relation to sleep apnea, J Am Geriatr Soc 1991, 39:258-263

28. Ancoli-Israel S, Kripke DF, Klauber MR, et al.: Periodic limb movements in sleep in community-dwelling elderly, Sleep 1991, 14:496-500

29. Tractenberg RE, Singer CM, Cummings JL, et al.: The Sleep Disorders Inventory: an instrument for studies of sleep disturbance in persons with Alzheimer's disease, J Sleep Res 2003, 12:331-337

Chapter 6

Strong association of the rest-activity rhythm with well-being in demented elderly women

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Abstract

Objective: To investigate the association between actigraphic estimates of the sleep-wake rhythm and a range of functional domains that contribute to well-being in demented elderly patients.
Method: Eighty seven women aged 85.5 ± 5.9 years (mean, ± SD) wore an actigraph for two weeks. Activity profiles were analysed using non-parametric variables including dichotomy indices, interdaily stability (IS), intradaily variability (IV) and relative amplitude (RA). The associations between these variables and cognitive, functional, behavioural and emotional states (obtained from standardized neuropsychological assessments and questionnaires administered to caregivers) were investigated by partial correlations and stepwise regressions.
Results: Cognitive, functional, behavioural and emotional states showed medium to large correlations with multiple rhythm variables. Partial correlations indicated that this could not be attributed to a uniform worsening with advancing cognitive decline. Stepwise regressions indicated three most distinctive rhythm variables: (1) the interdaily stability of the 24-hour rhythm was most strongly, negatively, related to cognitive decline and depression; (2) the median level of daytime activity was most strongly, negatively, related to impairments of function, of activities of daily living, and of social interaction; (3) nocturnal restlessness was secondarily, positively, related to impairments of function and of social interaction.
Conclusion: Especially the interdaily stability and median daytime activity level, and secondarily nocturnal restlessness, showed a strong relationship with the functional status and well-being of demented elderly. This raises the possibility that treatments that enhance daytime activity and the stability of the rest-activity rhythm may improve well-being.

Introduction

Age-related dementias are not only characterized by cognitive decline, but also by a range of non-cognitive symptoms. Of these, disturbances of the sleep-wake rhythm have convincingly been demonstrated[1-7] and are a major determinant of caregiver burden and, consequently, institutionalization. It is not fully understood, however, how the well-being of the patient is affected by sleep-wake rhythm disturbances.

Actigraphy is an objective and non-invasive method to estimate disturbances in human sleep-wake rhythms from rest-activity cycles measured in the field. The actigraph, a small and light device, is strapped onto the patient's wrist like a normal watch, and movements are recorded throughout the day and night without interfering with the patient's normal activities. As the 24-hour activity profile resembles more a square wave than a sinusoid, and because activity data are not normally distributed, non-parametric statistical methods instead of parametric methods (such as cosinor analysis) have been developed to quantify and analyse the activity trace. These methods make no assumptions about the profile of data distribution[8, 9].

One of these methods [8] assumes that the rest-activity rhythm has a periodicity of 24 hours and that two distinct periods of activity are expected to be observed during the course of a single 24-hour period: a sleep and a wakefulness period. The method consists of calculating two indices: The first dichotomy index, a measure of the percentage of activity counts obtained when the patient was out of bed that was greater then the median activity count when in bed, can be considered to be a "wake-active index" and is abbreviated to "WAI" in the present paper. Similarly, the second dichotomy index, a measure of the percentage of activity counts obtained when the patient was in bed that was less then the median activity count when out of bed, can be considered to be a "sleep-inactive index" and is abbreviated to "SII" in the present paper. A high value in either dichotomy index

indicates a more marked rest-activity rhythm, i.e., an active major activity period and restful major sleep period. The former index is more affected by activity changes during the major active period whereas the latter index is more influenced by activity during the major sleep period. The indices are ratios, thus correcting for possible confounding influences due to: unequal sensitivity of different actigraphs; differences between subjects or days of study in the exact placement of the device on the wrist; and the overall amount of activity of the subject. The method was applied for the first time to study the rest-activity rhythms of healthy subjects, a patient who suffered from delayed sleep phase insomnia, and of patients suffering from metastatic colorectal cancer[8]. Both dichotomy indices were used, and results showed that a lower dichotomy between activity in bed and activity out of bed was present in patients compared with healthy subjects. In later studies, dichotomy indices were applied in a field study to assess the rest-activity rhythm of nurses on night-work. The results obtained from the actigraphy record revealed that night-work (when sleep occurred during the day) was associated with lower dichotomies between activity in bed and out of bed in comparison with values during control/rest days (when sleep occurred during the night)[10].

Three other nonparametric variables have been proposed for quantification of the rest-activity rhythm: the Interdaily stability (IS), the Intradaily variability (IV) and the Relative amplitude (RA). The first variable gives an indication of the stability of the rhythm from day to day whereas the second gives an indication of the fragmentation of the rhythm, i. e. the frequency and extent of transitions between rest and activity. The third variable gives an indication of the amplitude of the rest-activity rhythm[11]. These calculations have often been applied to studies of the actigraphy record of dementia patients. For example, it has been observed that the stability of the rest-activity rhythm increased in dementia patients with intact vision after being exposed to bright light[11].

The aim of the present research was to investigate the association between different rest-activity parameters and disturbances in cognition, disease progress, competence to perform daily activities, mood and social interactions in dementia patients. Results will improve an understanding of the relationship between different rest-activity rhythm parameters and well-being in dementia patients.

Methods

2.1. Patients and facilities

Eighty seven women aged 85.5 ± 5.9 years (mean ± SD) were studied while living in assisted care facilities at 12 different homes for the elderly in The Netherlands. Participants were mostly demented elderly. The clinical diagnosis of dementia was made according to the DSM-IV criteria for dementia and dementia subtypes[12]. NINCDS-ADRDA criteria were used for the clinical diagnosis of probable Alzheimer's disease[13]. Of the 87 patients, 57 (66%) met the NINCDS-ADRDA criteria for probable Alzheimer's disease, 13 (15%) met DSM-IV criteria for Vascular dementia, 10 (11%) patients met criteria for other types of dementia. Five patients (6%) did not meet the criteria for dementia, but stayed in the group care facility for diverse medical or psychosocial reasons. In 2 (2%) patients, data about the medical history were insufficient to make a reliable clinical diagnosis.
To investigate the possibility that sleep-wake rhythms were systematically affected by the environmental setting of the patient, all care facilities were rated on the Therapeutic Environment Screening Scale (TESS)[14, 15]. The TESS assesses the quality of nursing home environments for residents with dementia and includes questions about the general conditions of the house such as noise, lighting, design, maintenance, as well as questions about staff interactions with residents and residents´ involvement in planned activities.

2.2. Procedure

Patients wore an actigraph (Actiwatch, Cambridge Neurotechnology, Cambridge, UK) for two weeks, strapped onto their non-dominant wrist. Activity was continuously recorded every minute while patients performed their habitual activities. The actiwatch was not removed when a bath was taken. The use of a specially designed strap made it difficult for the patients to remove the actigraph themselves. In case this still happened, the lost period was excluded from analysis. On average, valid data were available for 13.0 days ± 9 hours (mean±s.e.m.).

The following questionnaires were administered by the caregivers and neuropsychological assessments made by them in order to obtain the cognitive, functional, behavioral and emotional state of the patients, all of which are thought to contribute to the overall sense of well-being.

(1) Cognitive deficits were measured using the Mini-Mental State Examination Test (MMSE)[16] the higher the score, the less impaired is the patient's cognitive functioning. The MMSE focuses on cognitive decline and is obtained by direct questioning of the patient by a neuropsychologist;
(2) Functional impairment was assessed using the Functional Assessment Staging Scale (FAST) [17]–. The FAST separates the progressive deterioration of functional abilities into 16 stages – the higher the score, the more functional impairments the patient suffers. The FAST scores disease progression on the assumption of a monotonously increasing number of typical disabilities;
(3) Ability to perform daily activities was measured with the Nurse Informant Index of Activities of Daily Living (NI-ADL)[18, 19]. The NI-ADL is an adaptation of the Katz Index of Independence in Activities of Daily Living, optimized to obtain quantitative answers from nurse observants – the higher the score, the higher is the level of disability. The NI-ADL focuses on impairments of activities of daily living and is obtained through a questionnaire, i.e. indirect observations by caregivers;
(4) Mood was assessed using the Cornell Scale for Depression in Dementia (CSDD)[20]–the higher the score, the more symptoms of depression are present, and those that are present are more severe;
(5) Social interactions were evaluated using subscale V of the Multidimensional Observation Scale for Elderly Subjects (MOSES)[21]. The subscale consists of closed questions to quantify observations carried out in the preceding period on isolation and withdrawal behavior – a high score indicates a more severe lack of social interactions.

2.3. Data reduction and statistical analyses

To calculate dichotomy indices (WAI; SII), the hours of retiring and rising were determined for each 24-h section by visual inspection of the data. Day 1 was defined as the day when patients started to wear the actigraph, and the starting time for each 24-h section varied between 12:00 and 18:00 hours. The average dichotomy indices over all 24-hour sections were calculated for each patient.

Interdaily stability (IS), Intradaily variability (IV) and Relative amplitude (RA) were calculated from the activity profiles as follows.

1. IS: First, activity recording was aggregated in hourly bins counting the number of minutes containing any activity. Second, an average 24-hour activity profile was calculated by averaging each corresponding hour of all recorded days. IS is then calculated as the ratio between the variance of the average 24-hour pattern around the mean and the overall variance of the complete series of individual hourly averages. This yields a value between 0 and 1: the higher the value, the more predictable are the subsequent daily 24-hour patterns.

2. IV: First, differences between successive hourly counts of minutes containing any activity were calculated, and subsequently the variance over all these difference scores. IV is then calculated as the ratio of this variance of first derivatives and the overall variance of the complete series of individual hourly averages. The higher IV, the larger and/or more numerous are the transitions between rest and activity states.
3. RA: An average 24-hour profile on the minute-by-minute presence or absence of any activity was first calculated. Subsequently, average number of minutes with activity were calculated over two separate windows moving with one-minute steps through the 1440 (circular) one-minute averages.. One moving window was applied to determine the lowest mean activity during any stretch of 5 continuous hours (L5), another to determine the highest mean activity during any stretch of 10 continuous hours (M10). The relative amplitude RA was calculated as the difference between M10 and L5 divided by the sum of M10 and L5. This yields a value between 0 and 1; the higher the value, the larger was the difference between the major periods of rest and of activity.

To focus upon activity levels in bed and out of bed, the 50th percentile (median) of activity counts in bed (Sp50) and the 50th percentile (median) of activity counts out of bed (Wa50) were identified for each 24-hour period and the averages over all 24-hour sections were determined for each individual. High values for Sp50 indicate restless sleep, and low values for Wa50 indicate inactivity during the time awake. The measures L5 and M10, defined previously, were also used to study in more detail activity during the sleep and wake periods, respectively. The primary differences between L5/M10 and Sp50/Wa50 are that the former reflect the periods of least or most average activity limited to 5- or 10-h periods, whereas the latter quantify the median level of activity during the whole nocturnal and diurnal periods.

Kolmogorov – Smirnoff test indicated significant deviations from a normal distribution in L5, Sp50, Wa50, SII, and FAST and a trend for non-normality in the CSDD. To allow for partial correlations and multiple stepwise regressions, and to keep analyses uniform, all variables were normalized using the Blom transformation (SPSS 10, SPSS Inc., Chicago). Thus, normalized variables. were used to quantify the associations (Pearson correlation, partial correlation, stepwise regression) between the rest-activity parameters and the variables obtained from the questionnaires and neuropsychological assessments administered by the caregivers. Significance levels were set at $p \leq 0.05$.

Results

Examples of a very well maintained and very disturbed rest-activity are shown in Figure 1. Table 1 shows the uncorrected correlations of the normalized parameters. As to cognitive status, a higher level of cognitive functioning (MMSE) was associated with a more stable (IS) rest-activity rhythm. As to functional status, a more advanced functional impairment (FAST) and more difficulties in performing daily activities (NI-ADL) were associated with a more fragmented, less stable rest-activity rhythm (WAI,SII, IS, IV, RA), and with less daytime activity (Wa50, M10). A more advanced functional impairment was furthermore associated with a lower amplitude (RA). As to mood and social interaction, a higher number and severity of depressive symptoms (CSDD) was associated with a more fragmented, less stable and lower amplitude rest-activity rhythm (WAI, SII, IS, IV, RA), less daytime activity (M10) and more nocturnal activity (Sp50). A more severe social isolation was also associated with a more fragmented, less stable and lower amplitude rest-activity rhythm (WAI, SII, IS, IV, RA), as well as with less daytime activity (Wa50, M10).

To investigate whether disease progression underlied the rather uniform findings, partial

Table 1. Correlations between normalized parameters of rest-activity and well-being. All variables are in arbitrary units, except for M10 and L5,which quantify the average number of minutes per hour in which any activity is registered.

		Cognition	Functional Impairment	Difficulties with Activities of Daily Living	Mood Disturbances	Lack of Social Interactions
	mean ± s.e.	(MMSE) 14.8 ± 0.7	(FAST) 7.5 ± 0.23	(NI-ADL) 1.71 ± 0.12	(CSDD) 6.8 ± 0.62	(MOSES) 18.1 ± 0.72
Rest-activity rhythm						
WAI	69.2 ± 1.8		- 0.42 (<0.001)	- 0.42 (<0.001)	- 0.30 (0.007)	- 0.34 (0.002)
SII	76.8 ± 2.7		- 0.36 (0.001)	- 0.45 (<0.001)	- 0.38 (<0.001)	- 0.36 (0.001)
Interdaily Stability (IS)	0.65 ± 0.02	0.22 (0.04)	- 0.39 (<0.001)	- 0.39 (<0.001)	- 0.46 (<0.001)	- 0.39 (<0.001)
Fragmentation (IV)	0.74 ± 0.03		0.25 (0.02)	0.30 (0.006)	0.26 (0.02)	0.24 (0.03)
Amplitude (RA)	0.59 ± 0.02		- 0.26 (0.02)		- 0.26 (0.02)	- 0.34 (0.002)
Daytime activity						
Wa50	49.6 ± 5.7		- 0.43 (<0.001)	- 0.47 (<0.001)		- 0.39 (<0.001)
M10 (min/hr)	43.6 ± 1.1		- 0.37 (<0.001)	- 0.43 (<0.001)	- 0.22 (0.05)	- 0.37 (0.001)
Nocturnal activity						
Sp50	1.6 ± 0.6				0.24 (0.03)	
L5 (min/hr)	11.6 ± 0.7					

correlations were calculated, taking out the common contribution of the MMSE. The results, shown in Table 2, differ only marginally from the uncorrected correlations in Table 1, indicating that the relationships remain after correcting for the level of MMSE. Furthermore, given collinearity between some of the different sleep-wake rhythm parameters, stepwise regression analyses were run to obtain the rest-activity rhythm parameter(s) that were most strongly associated with the cognitive, functional and mood parameters. The results, shown in Table 3 and figure 2, show that worse scores on the MMSE and CSDD are most strongly related to a loss of day-to-day stability of the rhythm (IS), while social isolation (MOSES), functional impairment (FAST), and impairment of activities of daily living (NI-ADL) are most strongly related to a loss of daytime activity(Wa50). More severe functional impairment (FAST) and social isolation (MOSES) were secondarily related to increased nocturnal restlessness (L5). The cognitive, functional, behavioural and emotional states of the patients, as measured with the scales used in the present study, were thus only secondarily associated with nocturnal restlessness per se, and more strongly so with the daytime activity level and day-to-day stability in activity patterns.

No significant correlation was present of the environmental quality (TESS) with any of the sleep-wake rhythm parameters (average absolute r= 0.07, average p-value = 0.59). ANOVA's indicated no systematic differences of sleep-wake rhythm parameters in relation to the different diagnoses (average F=0.65, average p=0.71).

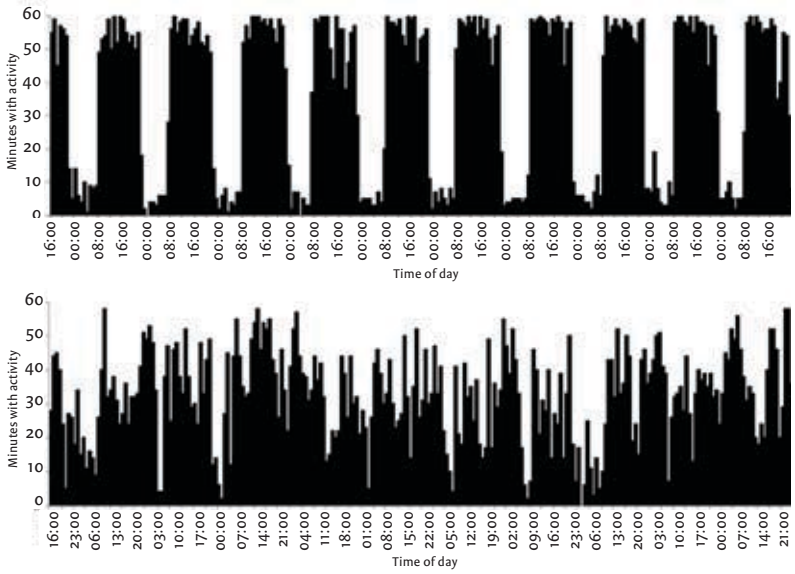

Figure 1
Examples of 14-day activity profiles of two patients. Bins represent the number of minutes with activity for each individual hour. The upper panel shows a subject with a very well-maintained rest-activity rhythm (IS=0.96, IV=0.29). Note especially the interdaily stability: the 14 individual daytime profile much resemble each other. The lower panel shows a subject with a virtually complete loss of 24-hour rhythmicity. Daily profiles do not resemble each other (IS=0.10) and the 'spiky' alteration of hours of much activity and little activity represent a strong fragmentation (IV=1.10).

Discussion

The major finding of the present study is a strong association of the activity profile with many aspects of functioning and well-being in demented elderly. In particular, the results indicate that primarily the 24-hour pattern of the rest-activity rhythm and the median daytime activity level, and secondarily the nocturnal restlessness, are the parameters most strongly associated with most functional domains that contribute to well-being in demented elderly patients.

The present study furthermore suggests those parameters that better describe the rest-activity rhythm disturbance in the patients studied. Limitations of function, of activities of daily living and of social interaction, as measured with the FAST, NI-ADL and MOSES respectively, are best reflected in a low Wa50, i.e. a low median daytime activity level. Nocturnal restlessness, quantified as the activity level in the most restful 5-hour period in the average activity profile, increases in parallel to the progression of functional impairments and social isolation (FAST and MOSES, Table 3). Depressive symptoms (CSDD) and cognitive decline (MMSE) are especially associated with a decrease in the day-to-day stability (IS) of the rest-activity rhythm. IS has previously been shown to enable a sensitive quantification of the rest-activity rhythm disturbances typical of dementia, and of the effect upon the rest-activity rhythm of light therapy[5, 9, 22]. Of note, a decrease in IS is not merely associated with increasing age, which rather affects fragmentation, nocturnal activity and the amplitude of the rhythm[23]. Also, the relations are not merely reflecting a generalized worsening merely due to disease progression, as indicated by the partial correlation analyses.

Studies in different cohorts of patients support the importance of a pronounced day-night rhythm. In patients with metastatic colorectal cancer, a marked rest-activity rhythm was associated with a better global quality of life (QoL) - particularly with better physical and social functioning, lower fatigue

Table 2. Partial correlations between normalized parameters of rest-activity and well-being after correction for disease progression (MMSE).

	Functional Impairment (FAST)	Difficulties with Activities of Daily Living (NI-ADL)	Mood Disturbances (CSDD)	Lack of Social Interactions (MOSES)
Marked rest-activity rhythm				
WAI	- 0.44 (<0.001)	- 0.40 (<0.001)	- 0.30 (0.008)	- 0.32 (0.004)
SII	- 0.37 (0.001)	- 0.44 (<0.001)	- 0.39 (<0.001)	- 0.35 (0.001)
Interdaily Stability (IS)	- 0.30 (0.007)	- 0.32 (0.004)	- 0.39 (<0.001)	- 0.33 (0.003)
Fragmentation (IV)	0.35 (0.01)	0.35 (0.001)	0.31 (0.005)	0.27 (0.02)
Amplitude (RA)				- 0.29 (0.009)
Daytime activity				
Wa50	- 0.42 (<0.001)	- 0.45 (<0.001)		- 0.37 (0.001)
M10 (min/hr)	- 0.37 (0.001)	- 0.41 (<0.001)		- 0.36 (0.001)
Nocturnal activity				
Sp50			0.24 (0.03)	
L5 (min/hr)				

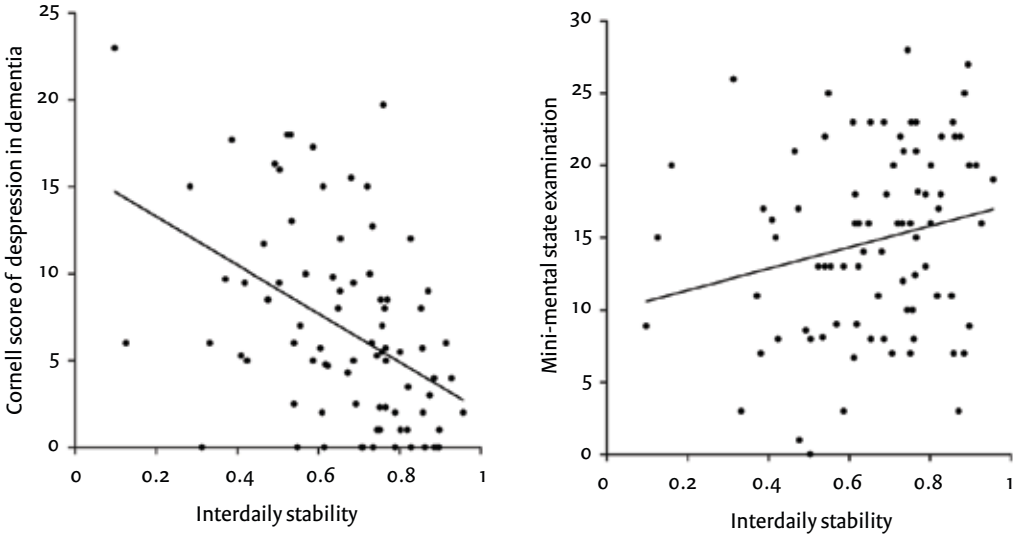

Figure 2
Scatterplots of the relation of the interdaily stability with (A) the Mini Mental State Examination (MMSE) and with (B) the Cornell Scale for Depression in Dementia (CSDD).

Table 3. Primary correlations between normalized parameters of rest-activity and well-being resulting from stepwise regression analysis.

	Cognition	Functional Impairment	Difficulties with Activities of Daily Living	Mood Disturbances	Lack of Social Interactions
	(MMSE)	(FAST)	(NI-ADL)	(CSDD)	(MOSES)
Marked rest-activity rhythm					
WAI					
SII					
Interdaily Stability (IS)	0.22 (0.04)			- 0.46 (<0.001)	
Fragmentation (IV)					
Amplitude (RA)					
Daytime activity					
W50		- 0.49 (<0.001)	- 0.47 (<0.001)		- 0.49 (<0.001)
M10 (min/hr)					
Nocturnal activity					
Sp50					
L5 (min/hr)		0.26 (0.009)			0.33 (0.003)

and appetite loss, less constipation and pain, and decreased intensity and frequency of symptoms of depression[24, 25].

In summary, our results indicate that the rest-activity rhythm is associated with a number of indirect indicators of the well being of demented elderly. Our correlational study does not allow for conclusions on causal directions between rest-activity rhythms. Neither can it be deduced from the present results if a poor circadian patterning of the periods of rest and activity are the result of a decreased functionality of the circadian timing system, including the suprachiasmatic nucleus, the biological clock of the brain. The rest-activity rhythm has a large exogenous component, as it t is masked by environmental demands on behaviour, and therefore it is less useful as a marker of the underlying circadian timing system. However, even if the disturbed activity patterns were caused wholly by factors other than changes in the circadian timing system itself, these disturbances would still affect this system. Thus, there is convincing evidence from both animal and human studies that both the level and the distribution of activity provide input to the circadian timing system[22, 26-29]. An irregular day-to-day activity profile may provide a sub-optimal, and possibly detrimental, input to the circadian timing system. This contention is supported by primate studies[30].

These results have important implications for other research on circadian rhythm disturbances in disease. Often, the focus has been on the amplitude and phase of the rhythms. Quantifying the median diurnal activity and particularly the repeatability of the activity record provides complementary information about the rest-activity changes associated with dementia. These variables may also be of value to research on rhythm disturbances in e.g. major depression or seasonal affective disorder.

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References

1. Gehrman, P. et al. The relationship between dementia severity and rest/activity rhythms. Neuropsychiatric Disease and Treatment 1, 155-163 (2005).

2. Harper, D. G. et al. Differential circadian rhythm disturbances in men with Alzheimer disease and frontotemporal degeneration. Arch Gen Psychiatry 58, 353-60. (2001).

3. Okawa, M. et al. Circadian rhythm disorders in sleep-waking and body temperature in elderly patients with dementia and their treatment. Sleep 14, 478-85 (1991).

4. Satlin, A., Volicer, L., Stopa, E. G. & Harper, D. Circadian locomotor activity and core-body temperature rhythms in Alzheimer's disease. Neurobiol Aging 16, 765-71 (1995).

5. van Someren, E. J. et al. Circadian rest-activity rhythm disturbances in Alzheimer's disease. Biol Psychiatry 40, 259-70. (1996).

6. Volicer, L., Harper, D. G., Manning, B. C., Goldstein, R. & Satlin, A. Sundowning and circadian rhythms in Alzheimer's disease. Am J Psychiatry 158, 704-11 (2001).

7. Tractenberg, R. E., Singer, C. M. & Kaye, J. A. Symptoms of sleep disturbance in persons with Alzheimer's disease and normal elderly. J Sleep Res 14, 177-85 (2005).

8. Minors, D. et al. The difference between activity when in bed and out of bed. I. Healthy subjects and selected patients. Chronobiol Int 13, 27-34 (1996).

9. Van Someren, E. J. et al. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int 16, 505-18 (1999).

10. Minors, D., Waterhouse, J., Folkard, S. & Atkinson, G. The difference between activity when in bed and out of bed. III. Nurses on night work. Chronobiol Int 13, 273-82 (1996).

11. Van Someren, E. J., Kessler, A., Mirmiran, M. & Swaab, D. F. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biol Psychiatry 41, 955-63 (1997).

12. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. (American Psychiatric Press, Washington, DC, 1994).

13. McKhann, G. et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34, 939-44. (1984).

14. Sloane, P. & Mathew, L. The therapeutic environment screening scale. Am J Alzheimers Care Related Disorders Res 5, 22-26 (1990).

15. Sloane, P. D. & Mathew, L. J. The therapeutic environment screening scale. American Journal of Alzheimer's Care and Related Disorders and Research 5, 22-6 (1990).

16. Folstein, M. F., Folstein, S. E. & McHugh, P. R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12, 189-98 (1975).

17. Reisberg, B. Functional assessment staging (FAST). Psychopharmacol Bull 24, 653-9 (1988).

18. Holmes, D. et al. Impacts associated with special care units in long-term care facilities. Gerontologist 30, 178-83 (1990).

19. Katz, S., Ford, A. B., Moskowitz, R. W., Jackson, B. A. & Jaffe, M. W. Studies of Illness in the Aged. The Index of Adl: A Standardized Measure of Biological and Psychosocial Function. Jama 185, 914-9 (1963).

20. Alexopoulos, G. S., Abrams, R. C., Young, R. C. & Shamoian, C. A. Cornell Scale for Depression in Dementia. Biol Psychiatry 23, 271-84 (1988).

21. Helmes, E., Csapo, K. G. & Short, J. A. Standardization and validation of the Multidimensional Observation Scale for Elderly Subjects (MOSES). J

Gerontol 42, 395-405 (1987).

22. Van Someren, E. J., Lijzenga, C., Mirmiran, M. & Swaab, D. F. Long-term fitness training improves the circadian rest-activity rhythm in healthy elderly males. J Biol Rhythms 12, 146-56 (1997).

23. Huang, Y. L. et al. Age-associated difference in circadian sleep-wake and rest-activity rhythms. Physiol Behav 76, 597-603 (2002).

24. Mormont, M. C. & Waterhouse, J. Contribution of the rest-activity circadian rhythm to quality of life in cancer patients. Chronobiol Int 19, 313-23 (2002).

25. Mormont, M. C. et al. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. Clin Cancer Res 6, 3038-45 (2000).

26. Turek, F. W. Effects of stimulated physical activity on the circadian pacemaker of vertebrates. J Biol Rhythms 4, 135-47 (1989).

27. Van Reeth, O., Zhang, Y., Reddy, A., Zee, P. & Turek, F. W. Aging alters the entraining effects of an activity-inducing stimulus on the circadian clock. Brain Research 607, 286-292 (1993).

28. van Someren, E. J., Mirmiran, M. & Swaab, D. F. Non-pharmacological treatment of sleep and wake disturbances in aging and Alzheimer's disease: chronobiological perspectives. Behav Brain Res 57, 235-53 (1993).

29. Buxton, O. M. et al. Roles of intensity and duration of nocturnal exercise in causing phase delays of human circadian rhythms. Am J Physiol 273, E536-42 (1997).

30. Cayetanot, F., Van Someren, E. J., Perret, M. & Aujard, F. Shortened seasonal photoperiodic cycles accelerate aging of the diurnal and circadian locomotor activity rhythms in a primate. J Biol Rhythms 20, 461-9 (2005).

Experimental studies

Chapter 7

Keep it bright: prolonged improvement of sleep and cognition in elderly residents of group care facilities by long-term combined light and melatonin administration

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Submitted

Abstract

Background: A good night's sleep sustains cognitive performance[1] while sleep-wake cycle desynchronisation induces learning deficits and reduced temporal lobe volume.[2, 3] Disturbed sleep, a frequent decisive factor in caregiver burden and institutionalisation[4] of Alzheimer patients, may thus augment their characteristic impairments. We hypothesized that a possibly reversible lack of activation of the circadian clock[5] could contribute to sleep problems in – mostly demented - elderly residents of group care facilities, and performed the first controlled human study on the effect of prolonged combined stimulation with light and melatonin.
Methods: During a long-term double-blind placebo-controlled follow-up study, 189 elderly received daily supplementation of the circadian synchronisers light (±1000 lux, whole-day), and/or melatonin (2.5 mg) for 15±1 months mean±s.e.m. on average and up to 3.5 years for a limited number of participants. Cognition (MMSE)[6] and actigraphic estimates of sleep-wake rhythms were assessed half-yearly.
Findings: Over time, combined light and melatonin administration continued to improve nocturnal restlessness by 9±3% per year ($p<0.01$), resulting in increased sleep duration and efficiency and a more pronounced amplitude of the 24-hour sleep-wake rhythm. Light improved cognition by 0.9±0.4 MMSE-points or 5% ($p=0.04$). ApoE-ε4 carriers had 20±9% longer nocturnal awakenings ($p=0.03$), while the sleep duration of ApoE-ε2 carriers improved 8±4% stronger in response to combined treatment ($p<0.05$). Previously suggested adverse reactions to melatonin and especially light occurred less rather than more in the active treatment conditions.
Interpretation: This first study on long-term stimulation of the human circadian timing system showed effects of prolonged combined melatonin and whole-day bright light treatment in elderly residents of group care facilities that may be clinically relevant. Improvement of the sleep-wake rhythm contributed to attenuation of cognitive decline, with an effect size comparable to that achieved with acetylcholinesterase inhibitors in a younger and more homogeneous group of Alzheimer patients.[7] Further novel findings are the interaction effects of light and melatonin, and that some of these take months to fully develop.

Introduction

In the absence of light, endogenous circadian rhythms may free run with a period deviating from 24-hours. In order to keep these rhythms, including the sleep-wake rhythm, synchronised with the environmental 24-hour light-dark cycle, daily resetting is required.[8] Both light and melatonin are strong synchronisers (Zeitgeber) of suprachiasmatic nuclei (SCN) activity and its output. Post-mortem investigation of the human SCN revealed a decrease in the number of vasopressin immunoreactive neurons and vasopressin-mRNA levels with aging with an exaggerated decline at a younger age in demented elderly.[5, 9] Since vasopressin expression is considered to be a marker for the output strength of the SCN as a circadian pacemaker[10], these deficits may contribute to the sleep-wake rhythm disturbances that occur so frequently in demented elderly.[11]

In very old rats, an increase in daytime light intensity – providing a strong Zeitgeber input to the SCN - restored the age-related decrease in vasopressin expression as well as the sleep-wake rhythm amplitude.[12, 13] In demented elderly we could show that the severity of rest-activity rhythm disturbances was associated with a lack of light exposure[14], and that rhythms improved after 4 weeks of increased daytime illumination.[15] These findings suggest that even in elderly residents of group care facilities the output of the circadian timing system can be enhanced by the appropriate stimulus, but long-term controlled studies have not previously been performed.

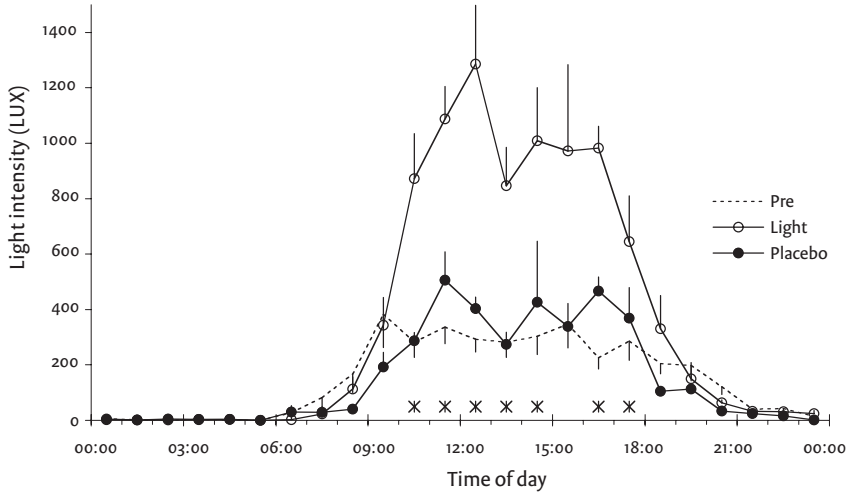


Figure 1
Average 24-hour light exposure profiles before (dashed line) and after installing the active (open circles) and placebo (closed circles) lights. Individual lux measurements (Mavolux Digital, Gossen, Nürnberg, Germany) were done at eye level in the direction of gaze, i.e. usually quantifying slightly downward or at best vertical illumination levels, which are considerably lower but more adequate than horizontal assessments directed towards the light sources. Assessments include observations made if subjects were not actually present in the common living room where the lights were installed, but in their own bedroom. Asterisks indicate the hours of significantly increased light intensity in the active condition ($p \leq 0.01$). None of the hours showed increased intensity in the placebo condition. Mean ± s.e.m. values were obtained from multilevel analysis of 3017 light measurements from 189 subjects in 12 facilities obtained over up to 3.5 years throughout the 24-hour day.

Experimental studies in healthy subjects and animals indicate that sleep supports daytime cognitive performance, notably memory consolidation and executive functions.[1, 16] In Alzheimer's disease (AD) patients, an association of their frequently occurring sleep disturbances with cognitive performance and functional status has also been reported.[17] This raised the possibility that amelioration of disturbed sleep-wake rhythms might improve cognitive performance within the limits set by the stage of the disease.

We investigated the effects of long-term daily supplementation of bright light (±1000 lux, whole-day, see Figure 1) and/or melatonin (2.5 mg) on sleep and cognition in a controlled study on 189 institutionalised, mostly demented, elderly followed up every 6 months for 15±1 months mean±s.e.m. on average and up to 3.5 years for a limited number of participants. The effects on non-cognitive symptoms and circadian rhythms in core temperature, melatonin and cortisol were evaluated as well and will be reported separately.

We included an evaluation of the involvement of ApoE genotype in the presence and development of disturbed sleep and treatment responses. Cacabelos et al.[18] found sleep disorders to occur more frequently in ApoE-ε3/4 carriers than in ApoE-ε4/4 carriers. Yesavage and collegues[19] followed 40 AD patients during the course of their illness and reported an accelerated worsening in sleep in patients negative for the ε4 allele. No prior studies evaluated involvement of ApoE genotype in sleep treatment responses.

Materials and methods

Subjects and group care facilities

Subjects (189), aged 85.8±0.4 years (mean±s.e.m.), mostly female (170), were residents of group-care facilities of 12 Dutch homes for the elderly, where subjects have their own apartment where they sleep and retreat, but spend most of the daytime in

a common living room. Clinical diagnoses in this heterogeneous group were, according to consensus criteria[20-22], probable AD (120), Vascular dementia (20), Frontal type dementia (3), Lewy body dementia (2), Parkinson's disease (2), Wernicke-Korsakoff (1), Dementia due to Multiple Etiologies (9), and Dementia not otherwise specified (7). Seventeen subjects did not meet criteria for dementia, but stayed in the group-care facility for medical or psychosocial reasons. In the remaining 8 subjects, the medical history data were insufficient to make a reliable clinical diagnosis. Prior to installation of the light fixtures, the overall quality of the group care facilities was rated using the Therapeutic Environment Screening Scale (TESS-2+).[23, 24] The Medical Ethical Committees of Hospital De Gelderse Vallei, Ede and the VU University Medical Center, Amsterdam, The Netherlands, approved the study, and subjects only participated after informed consent was given by their relatives.

Study Design

Subjects were randomly and double blind assigned to receiving a daily dose of melatonin (n=95, 2.5 mg, Terafarm, Brielle, The Netherlands) or placebo (n=94), 1 hour before bedtime. The tablets took about one hour to dissolve. Facilities were randomly assigned to active (6 facilities, n=98) or placebo (6 facilities, n=91) light exposure. All randomization was done by a research assistant not involved in the study (J. van Heerikhuize, NIN), and kept confidential until completion of the study. Treatment times were chosen to mimic the normal patterns of nocturnal melatonin surge and diurnal light exposure. Light was delivered by installing ceiling-mounted fixtures with Plexiglas diffusers containing an equal amount of Philips TLD 840 and 940 fluorescent tubes, lit daily between ± 9:00 and 18:00 hr. We aimed at an exposure of ±1000 lux, measured before the eyes in gaze direction. Such daytime light intensity has in previous studies been shown to synchronize circadian rhythms in healthy subjects in temporal isolation[25] and to improve circadian activity rhythm disturbances in moderately to severely demented elderly.[15] Moreover, the latter study indicated that an exposure of ±1000 lux is both feasible and tolerated in most group care facilities, while higher intensities would take considerably more technical effort. In the placebo condition an equal amount of fixtures were installed, but they contained only half the tubes, accommodated concealed band-stop filters and were at a larger distance from the eyes. The average light exposure measured at eye-level in the gaze direction is shown in Figure 1. Patients and caregivers were blind to the light treatment condition of their facility since they were not informed and never able to compare light installations between active and placebo light locations. The level of cognitive functioning of the patients was too low to explain about the placebo-controlled study and obtain a valid estimate of their appreciation of the light installation. Blindness of the caregivers to the light treatment condition of their facility was verified by a lack of difference between 184 ratings obtained from 89 caregivers over the treatment period on an "illumination pleasantness" visual analogue scale (t-test, two tailed, p=0.47) and on the odds of a confirmative answer to the question whether they thought their facility had effective light (chi-square test, two tailed, p=0.62).

The study lasted 3.5 years in which subjects were on average followed up for 15±1 months. In 129 subjects, assessments of sleep-wake rhythms and cognition were made 6 weeks prior to and 6 weeks after the start of the treatment (for short-term effect evaluation), and subsequently every 6 months with a maximum of 3.5 years. Another 60 subjects were enrolled in the study at later time points: lights had already been installed before their admission to the care facilities (see also part on statistical power below).

After enrolment, a subject's maximal follow-up period was primarily determined by the duration of participation of their facility. Due to logistical reasons including rebuilding, moving and staff limitations, participation of facilities varied between 3.5 years (four facili-

ties); 3 years (one facility); 2.5 years (two facilities);); 2 years (two facilities); 1.5 year (two facilities) and 0.5 year (one facility). Secondarily, a major number of subjects were lost from follow-up assessment due to death or outplacement to a nursing home. Figure 2 A. shows the cumulative frequency distribution of the percentage of subjects available for observation and the percentage lost due to retreat, nursing home placement or death. Figure 2 B. shows a flow chart of subjects included in the study.

On each assessment occasion, estimates of sleep were obtained from 14±4 (minimum 3) days of actigraphic measurements (Cambridge Neurotechnology, Cambridge, UK), analysed with validated software[26], using habitual bedtime and get-up time provided by the nursing staff. The calculated sleep variables quantify three processes of sleep: (1) the quantity of sleep as expressed in its duration, onset latency and efficiency (percentage asleep of time in bed); (2) the within-sleep structure as expressed in the duration of nocturnal awakenings and of uninterrupted periods of sleep; (3) the 24-hour structure of the sleep-wake cycle, as expressed in the inter-daily stability (IS) or the inter-daily predictability of the 24-hour pattern, intra-daily variability (IV) or the fragmentation of periods of rest and activity, the minutes per hour containing activity during the most restful five hour period of the average 24-hour pattern (L5), and the relative amplitude (RA), i.e. the relative difference in active minutes per hour between the most active ten hour period (M10) and L5.[27] The following endpoints were established for each class of sleep evaluation: sleep efficiency as a proxy for sleep quality; the duration of uninterrupted periods of sleep as a proxy of sleep depth and L5 to quantify nocturnal restlessness. The rest-activity rhythm amplitude (RA) was determined as the most comprehensive variable of interest to investigate circadian mechanisms involved in possible treatment effects.

The endpoint for cognitive performance was the Mini Mental State Examination (MMSE).[6] The MMSE was determined by transferring the patient from the facility to a separate office where a score was obtained by a trained neuropsychologist blind to the treatment condition. The MMSE score ranges from zero in case of minimal performance to a maximal performance of 30.

Adverse effects evaluation

At each assessment, caregivers were asked to rate each subject on the occurrence during the last two weeks of possible adverse reactions as have been suggested in previous studies on light or melatonin treatment. The rating was given on a 16-item 4-point scale (0=absent, 1=probably absent, 2=probably present, 3=present). At the onset of the study, the patients' physicians were asked to report any suspicion of severe adverse events related to the treatment.

ApoE Genotyping

ApoE genotype was obtained to evaluate in ancillary analyses its possible modulation of treatment effects.[28] A detailed description of the procedure for determining the ApoE genotype from saliva is available from the authors.

Data analysis and statistics

Mixed-effects regression analysis[29] (MLwiN, Institute of Education, London, UK) was applied to test for the significance of main and time-dependent effects of light, melatonin and their interaction. This method allows the use of all available and quite variable number of observations of each subject, without the need of imputation. The regression models included random intercepts to take into account the three-level nested and correlated data structure, i.e. a variable number of observations i nested within subjects j, and subjects grouped in 12 facilities k. This approach results in separation of the residual error variances at the levels of facility, subject and observation, which are for reasons of parsimony not shown in table 2.

Because previous studies on the effect of light and melatonin have been of limited duration, they have not allowed for

conclusions on whether light or melatonin treatment would remain efficacious when applied for a longer term than just a few weeks. Effect might as well fade out, or the reverse, grow slowly over months of treatment. Therefore, analyses were planned to evaluate both (1) treatment-effects that were immediate and of which the effect size did not change over time in treatment, as well as (2) time-by-treatment effects, i.e. with an effect size changing over time in treatment. Melatonin, light and their interaction were dummy coded in three variables indicating the presence of active treatment at any observation, i.e. 1 in case of active treatment and 0 for all observations prior to treatment onset and in case of placebo treatment. Given the longitudinal character of the dataset, 'time' was included in the model as a discrete factor. In the analyses, special attention was given to the fact that- especially after 1.5 years - many cases were lost from follow-up either due to noninformative reasons (discontinuation of participation of the facility) or to possibly informative causes. First, in order to obtain the most simple acceptable regression equation insensitive to a reduction in the follow-up time, we verified whether treatment effects obtained from analyses on the complete 3.5 year dataset were still present in a reduced dataset including only the first 1.5 year of follow-up. A second approach was to code missing data due to (1) death or nursing home placement, or (2) non-compliance or insufficient communication abilities, in two dummy variables (indicating presence of this condition for a subject at any point in time) to allow for inclusion in the regression analysis according to a pattern mixture model approach.[30] Thus, the initial full model multilevel regression equation fitted to the data was of the form:

$$\begin{aligned}\text{Outcome}_{ijk} = {} & \beta_{0ijk} + \beta_1 * \text{Light}_{ijk} \\ & + \beta_2 * \text{Melatonin}_{ijk} \\ & + \beta_3 * \text{Light} * \text{Melatonin}_{ijk} \\ & + \beta_4 * \text{Time}_{ijk} \\ & + \beta_5 * \text{Time} * \text{Light}_{ijk} \\ & + \beta_6 * \text{Time} * \text{Melatonin}_{ijk} \\ & + \beta_7 * \text{Time} * \text{Light} * \text{Melatonin}_{ijk} \\ & + \beta_8 * \text{MissingPattern1}_{ijk} \\ & + \beta_9 * \text{Time} * \text{MissingPattern1}_{ijk} \\ & + \beta_{10} * \text{MissingPattern2}_{ijk} \\ & + \beta_{11} * \text{Time} * \text{MissingPattern2}_{ijk}\end{aligned}$$

where outcome is the (sleep or cognition) variable of interest and each observation in time is denoted as i, each subject as j, and each facility as k. The betas provide the intercept (β_0) and effect estimates (β_1 to β_{11}) The regression equations were re-evaluated after each step of the stepwise exclusion of the least significant terms, of which the exclusion did not significantly increase the residual error of the equations according the −2loglikelihood ratio chi-square test with a two-tailed significance level set at 0.05.[27] The resulting most simple acceptable regression equations thus included only variables with significant effect sizes. It was evaluated post-hoc whether the resulting significant treatment effects were modulated by any of the missing patterns and whether level, time course or treatment effects were modified by Alzheimer's diagnosis (dummy coded 0-1) and by ApoE genotype (two dummy codes, respectively for presence of least one ApoE-ε2 or at least one ApoE-ε4 allele).

In further ancillary analyses, the relationship between sleep and cognitive performance was investigated in three ways, once more applying mixed effects regression analyses to account for the variable number of observations nested within subjects, and subjects grouped in 12 facilities. The first set of analyses addressed the question whether MMSE ratings related to simultaneously obtained sleep parameters. In order to account for possible confounding, i.e. spurious relations resulting from simultaneous changes over time or in association with dropout pattern, the latter variables and their interaction were included as covariates in the regression equations. In its general form, the initially fitted equation was as follows:

$$MMSE_{ijk} = ß_{0ijk} + ß_1*SleepVariable_{jik} + ß_2*Time_{ijk} + ß_3*DropoutPattern1_{ijk} + ß_4*Time*DropoutPattern1_{ijk} + ß_5*DropoutPattern2_{ijk} + ß_6*Time*DropoutPattern2_{ijk}$$

Nonsignificant terms were removed stepwise as described before.

The second set of analyses addressed the question whether the rate of MMSE decline can be predicted from simultaneously occurring alterations in sleep. Rate of change (ROC) scores were calculated for all MMSE and sleep scores for which a preceding observation was available. In its general form the regression equation was as follows:

$$ROCMMSE_{ijk} = ß_{0ijk} + ß_1*ROCSleepVariable_{ijk}$$

Note that the intercept $ß_{0ijk}$ in this equation represents the estimated rate of change in MMSE in absence of the possible contribution $ß_1$ due to changes in the sleep variable under consideration.

The third set of analyses addressed the question whether treatment-induced changes in sleep variables contributed to treatment effects on cognitive performance. Multilevel mediation analyses[31] were used to investigate the possibility that treatment effects on cognitive performance were in part mediated by their effects on sleep. In brief, the mediation analysis applied a fourth regression analysis if three regression analyses (already reported above) showed that treatment affected both the MMSE and the sleep variable under study, and that there was predictive value of the sleep variable for the MMSE. The additional fourth analysis evaluated the predictive value of the sleep variable on the MMSE after partialing out the effect of treatment on the MMSE. In case of significant effects in all four regression analyses, (partial) mediation is supported, and its significance can be tested.[31]

Statistical power

At the onset of the study it was estimated that subjects would on average remain in the protocol for 2.5 years, allowing for 6 follow-up assessments: one short-term and five half-yearly. Under the assumption of a within-subject correlation of r=0.50 and using the formulas provided by Twisk,[29] 147 subjects would be needed to attain, at an alpha of 0.05, a power of 0.80 to detect effect sizes of d=0.25 i.e. between the conventional definition of a small (0.20) to moderate (0.50) effect size. The absolute minimal aim was set at 140. Since new inhabitants, assigned to the special care facilities after onset of the study, were faced with the presence of the dedicated lighting systems, it was determined at the onset of the study that they were to be given the possibility to participate, both because of ethical considerations and because of statistical power considerations: power and smallest detectable difference calculation outcomes vary with small deviations from estimated sample size, effect size or within-subject correlation. For these reasons, an unlimited number of subjects were allowed to enter the study until it finished. The longest duration of data-acquisition in a single facility was set at 3.5 years after start of treatment.

Results

Enrolment commenced in June 1999 and data acquisition continued until April 2004. Twelve homes for the elderly participated, in which forty-nine subjects were assigned to light only, 46 to melatonin only, 49 to their combination and 45 to double placebo. The ratio of subjects assigned to the active melatonin condition within each facility was 0.50±0.02 (mean±s.e.m.), indicating successful balancing of the randomization procedure. Figure 2 provides an overview of the subjects included and followed-up in the four conditions. Table 1 shows that randomisation turned out to be balanced, in that none of the subject characteristics, environmental characteristics or pre-treatment outcome variable levels differed significantly between the four groups (all p>0.05, Chi-square tests on frequencies, ANOVAs on levels).

Over the years, a neuropsychologist blind

to the condition applied the mini-mental state examination (MMSE)[6] to obtain a total of 619 succesful cognitive assessments, of which 500 follow-up assessments, from 174 subjects. Of the maximum number of observations (744), 15% failed due to non-compliance or insufficient communication abilities and 1% due to logistics. The structure and quantity of sleep and the 24-hour pattern of rest and activity were estimated from 566 14(±4)-day succesful actigraphic recordings, of which 466 follow-up assessments, from 164 subjects.[32] Of the maximum number of observations (744), 22% failed due to non-compliance and 2% due to logistics.

Cognitive decline

Regression analyses showed the cognition ratings (MMSE) to have an intercept of 17.0±1.0 points, to be lowered by 3.5±0.8 points for those subjects who dropped out of the study due to nursing home placement or death (Table 2 and Figure 3). The MMSE decline over time was 1.1±0.2 points per year, and an additional 1.5±0.3 in the drop-out subjects. Light attenuated deterioration of the MMSE, by 0.9±0.4 points or 5% (all % given relative to intercept) (CI (95% confidence interval) 0.04-1.71, p=0.04), i.e. better described as a fixed difference at all time points than as affecting the rate of decline.

Sleep

Light and melatonin treatment affected sleep in several ways (Table 2). As to *sleep quantity*, the three variables obtained were all affected by the treatments. Combined treatment (light and melatonin) increased sleep efficiency by 3.5±1.8% as compared to an efficiency of 73±1% (CI 0.84-6.09 %SE; p=0.01) for assessments made under only one or no active treatments. Melatonin shortened sleep onset latency by 8.2±3.6 minutes or 19% as compared to an unchanging latency of 43±3 minutes (CI 1.1-15.4 min, p=0.02) for assessments made in absence of active melatonin treatment. Sleep duration increased by 27±10 minutes (6%) during melatonin treatment (CI 9-46 min, p=0.004) and by 10±5 minutes (2%) per year during light treatment (CI 0.4-20 min; p=0.04) as compared to an unchanging duration of 7 hr and 51±12 minutes for assessments made under only one or no active treatments. Only in the 3.5 year regression analysis, but not confirmed in the analysis on the limited dataset, ApoE-ε2 carriers receiving combined treatment showed an additional increase in sleep duration of 37±19 minutes (8%) per year (CI 0.8-73 min; p<0.05).

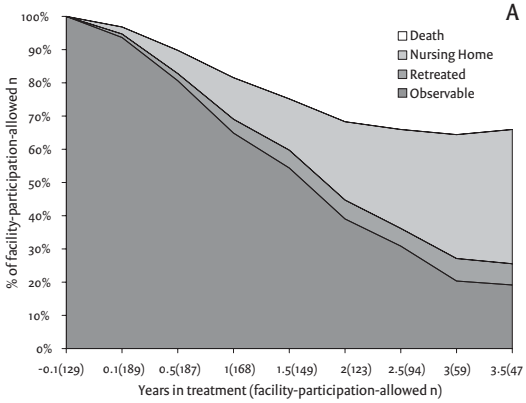
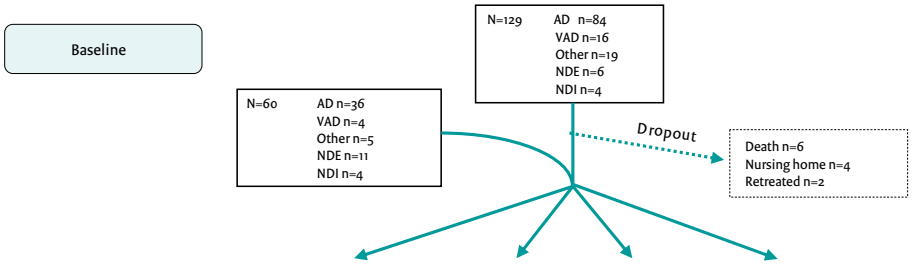


Figure 2

A. Cumulative frequency distribution of the percentage of subjects available for observation and the percentage lost due to retreat, nursing home placement or death. At each assessment point (horizontal axis) the percentage is expressed relative to the facility-participation allowed maximal number of subjects had everybody still been observable (given in brackets at the horizontal axis). Note that, of the observable cases, a percentage of assessments missed due to logistics, non-compliance or insufficient communication abilities (see text for numbers).
B. Flow chart of subjects included in the study. One hundred twenty nine subjects started with the pre-treatment assessment (AD = Alzheimer's disease, VAD = vascular dementia, other = other types of dementia, NDE = not demented, NDI = not diagnosed due to lack of data). Sixty subjects entered the study later (see text) and are included in the chart from post-treatment assessment 1 onwards, irrespective of the time relative to their facilities duration of participation. Reasons for dropout were death, nursing home placement, retreated informed consent or study end of the facility. The first post-treatment assessment 1 was 6 weeks after the start of the light and/or melatonin treatment, assessment 2 half a year after the start of light and/or melatonin and the subsequent assessments were done every next half year.

B



Post-treatment 1 6 wk	Light & Melatonin Total n=43	Light Total n=48	Melatonin Total n=46	Placebo Total n=40
	31 AD 6 VAD 3 Other 3 NDE 0 NDI	36 AD 5 VAD 6 Other 1 NDE 0 NDI	28 AD 3 VAD 6 Other 5 NDE 4 NDI	20 AD 4 VAD 7 Other 8 NDE 1 NDI

Post-treatment 2 6 months	N=150	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatonin	36		4	1	0	2
Light	44		2	2	0	0
Melatonin	39		2	3	1	1
Placebo	31		5	3	1	0

Post-treatment 3 1 year	N=109	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatonin	26		1	5	0	4
Light	34		0	3	0	7
Melatonin	27		7	3	0	2
Placebo	22		5	1	3	0

Post-treatment 4 1.5 years	N=81	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	21		1	1	0	3
Light	24		4	2	0	4
Melatonin	18		4	2	0	3
Placebo	18		2	0	1	1

Post-treatment 5 2 years	N=48	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	12		4	3	0	2
Light	17		3	2	0	2
Melatonin	9		3	2	0	4
Placebo	10		1	2	1	4

Post-treatment 6 2.5 years	N=29	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	9		2	0	0	1
Light	14		1	1	0	1
Melatonin	4		0	2	0	3
Placebo	2		2	1	0	5

Post-treatment 7 3 years	N=12	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	3		0	2	0	4
Light	5		0	2	0	7
Melatonin	2		0	0	0	2
Placebo	2		0	0	0	0

Post-treatment 8 3.5 years	N=9	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	3		0	0	0	0
Light	3		1	0	0	1
Melatonin	2		0	0	0	0
Placebo	1		0	1	0	0

Table 1. Subject characteristics, environmental characteristics and pre-treatment outcome variable levels for the four treatment groups. The table shows counts per group and group means ± standard deviations. None of these differed significantly (all p>0.05, Chi-square tests on frequencies, ANOVAs on levels).

Parameters	Double Placebo	Light Only	Melatonin Only	Combined
Group Characteristics (N=189)				
N	45	49	46	49
Females	40	45	38	47
Deceased	18	12	16	14
Outplaced	9	13	12	14
Alzheimer's diagnosis	22	37	28	33
Other diagnosis	23	12	18	16
ApoE-ε 2 allele present / subjects characterized	2/19	2/26	4/31	4/27
ApoE-ε 4 allele present / subjects characterized	5/19	5/26	7/31	6/27
Age at first assessment (yr)	85 ± 5	85 ± 6	86 ± 5	87 ± 6
Date of first assessment	11-May ± 85 days	9-Jun ± 62 days	12-May ± 85 days	7-Jun ± 70 days
Days followed up	381 ± 343	550 ± 389	433 ± 324	443 ± 393
TESS	104 ± 9	101 ± 10	104 ± 8	102 ± 12
Pre-treatment values (N=129)				
Sleep quantity				
Sleep efficiency (%)	76 ± 13	70 ± 16	72 ± 13	73 ± 11
Sleep onset latency (min)	31 ± 23	50 ± 36	54 ± 61	44 ± 34
Total sleep duration (hour:min)	8:42 ± 2:04	7:26 ± 2:06	8:18 ± 1:31	7:44 ± 1:24
Sleep structure				
Mean duration of intermittent awakenings (min)	4.4 ± 2.5	5.1 ± 2.8	4.3 ± 1.6	4.8 ±2.2
Mean duration of uninterrupted sleep periods (min)	33 ± 38	23 ± 12	18 ± 7	25 ± 15
Sleep-wake 24-hour pattern				
Interdaily Stability (IS)	0.57 ± 0.19	0.68 ± 0.15	0.65 ± 0.22	0.67 ± 0.17
Intradaily Variability (IV)	0.80 ± 0.31	0.79 ± 0.33	0.70 ± 0.31	0.77 ± 0.31
Activity in least active five hours (L5, min/hour)	11.1 ± 6.5	12.6 ± 9.5	11.8 ± 6.1	11.4 ± 5.3
Relative Amplitude (RA)	0.57 ± 0.19	0.60 ± 0.19	0.57 ± 0.17	0.59 ± 0.15
Cognition				
MMSE	14.3 ± 7.0	14.5 ± 6.2	15.3 ± 5.3	14.7 ± 6.8

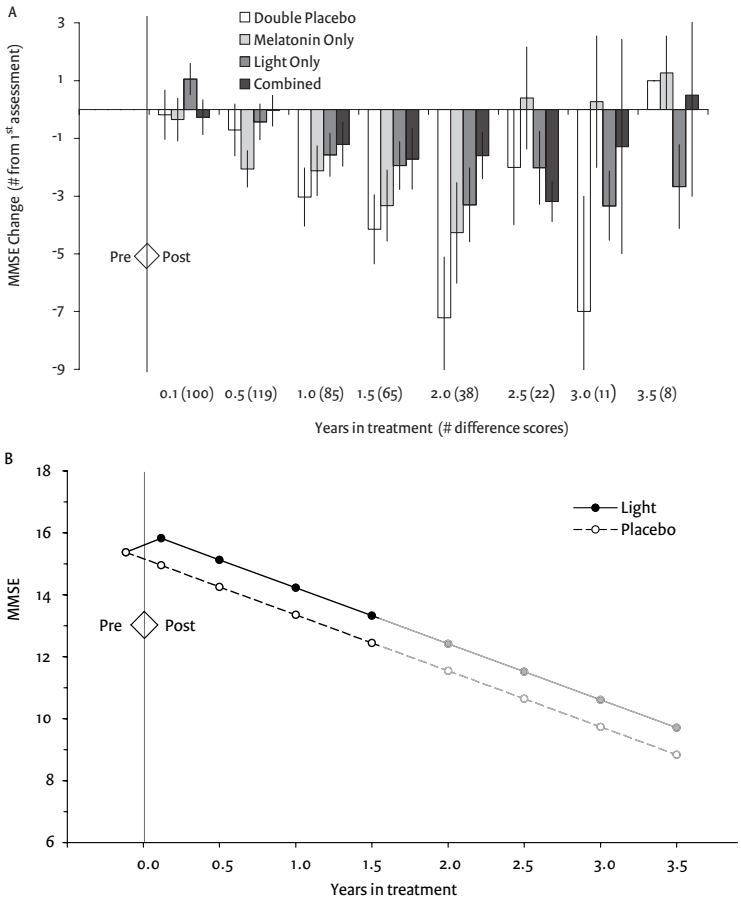


Figure 3.

MMSE-change group averages per time point and graphical representation of the regression parameters for cognitive decline. **A.** Changes in MMSE ratings (mean ± s.e.m.) relative to the first assessment four each group per follow-up assessment. Note the decline in number of observations (n, given between brackets on the horizontal axis) over the study period of three and a half years. Since the values obtained after 2 years of treatment are based on only a very limited number of subjects still included, they should be regarded of little value – as also indicated by the large standard errors. Up to 2 years of treatment, the decrease in MMSE is less in the subjects in the active light condition (hatched bars). Note that there also appears to be an attenuation of decline in the subjects in the active melatonin condition, which becomes evident only by the 1 year follow-up assessment, suggestive for a very slow onset melatonin efficacy. Indeed, in the full dataset, there was an independent additive effect of melatonin, which did attenuate the MMSE decline, best described by a treatment by time interaction effect of 0.6±0.3 or 3% per year (CI 0.02-1.14, p=0.04). However, this effect could not be confirmed in the verification analysis on the restricted dataset of 1.5 years. **B.** Graphical representation of the most simple acceptable regression model for cognitive decline as assessed by the MMSE. Given that the majority of data were collected within the first one a half year of treatment, the estimated effects after this period should be interpreted with caution, as indicated by the shading of the lines. Light and/or melatonin treatment commenced at time zero. Treatment started 6 weeks after the first assessment and 6 weeks prior to the second. As compared to placebo (dotted line), bright light (solid line) induced a relative increase, independent of time in treatment. On the other hand, the effect of melatonin administration (filled circles) as compared to placebo (open circles) developed slowly over time. The multilevel regression equation shown is: $MMSE_{ijk} = ß_{oijk} + ß_1 * Time_{ijk} + ß_2 * DropoutPattern1_{ijk} + ß_3 * Time * DropoutPattern1_{jk} + ß_4 * Light_{ijk}$ for each observation i in subject j of facility k. Effect coefficients are given in the text and table 2. All curves show the weighed averages for subjects that dropped out of the study due to nursing home placement or death (contributing 49% of the data) and for subjects that provided assessments as long as their facility participated (51% of the data).

Table 2. Regression effect estimates ± s.e.m. Outcome variables are listed in the left column. The second column gives the regression intercepts. Columns three to five show, respectively, the rate of change per year and the difference in level and rate of change for those subjects that left the study due to death or nursing home placement. The next 3 columns show the significant ($p<0.05$) treatment effects for Light, Melatonin and their interaction ('Combined'). The rightmost 3 columns show the treatment by time effects, given as rate of change per year. Blank cells indicate absence of significant (additional) effects. L5 = most restful five-hour period of the average 24-hour pattern, RA = relative amplitude, MMSE = mini mental state examination.

	Intercept	Rate of change	Dropout	Rate of change	Treatment effects			Treatment by time effects (/year)		
Parameters				by Dropout	Light	Melatonin	Combined	Light	Melatonin	Combined
Sleep quantity										
Sleep efficiency (%)	73 ± 1						3.5 ± 1.3			
Sleep onset latency (min)	43 ± 3					-8.2 ± 3.6				
Total sleep durtion (hour:min)	7:51 ± 0:12					+00:27 ± 0:10		+00:10 ± 0:05		
Sleep structure										
Mean duration of intermittent awakenings (min)	4.3 ± 0.2									-0.5 ± 0.2
Mean duration of uninterrupted sleep periods (min)	24 ± 2					+5.8 ± 2.4				
Sleep-wake 24-hour pattern										
Interdaily Stability (IS)	0.67 ± 0.02		-0.07 ± 0.03	-0.05 ± 0.01						
Intradaily Variability (IV)	0.74 ± 0.03		0.10 ± 0.05	0.07 ± 0.02						
Activity in least active five hours (L5, min/hour)	12 ± 0.6									-1.0 ± 0.4
Relative Amplitude (RA)	0.58 ± 0.02			-0.04 ± 0.01						+0.03 ± 0.01
Cognition										
MMSE	17.1 ± 1.0	-1.4 ± 0.2	-3.5 ± 0.8	-1.5 ± 0.3	+0.9 ± 0.4					

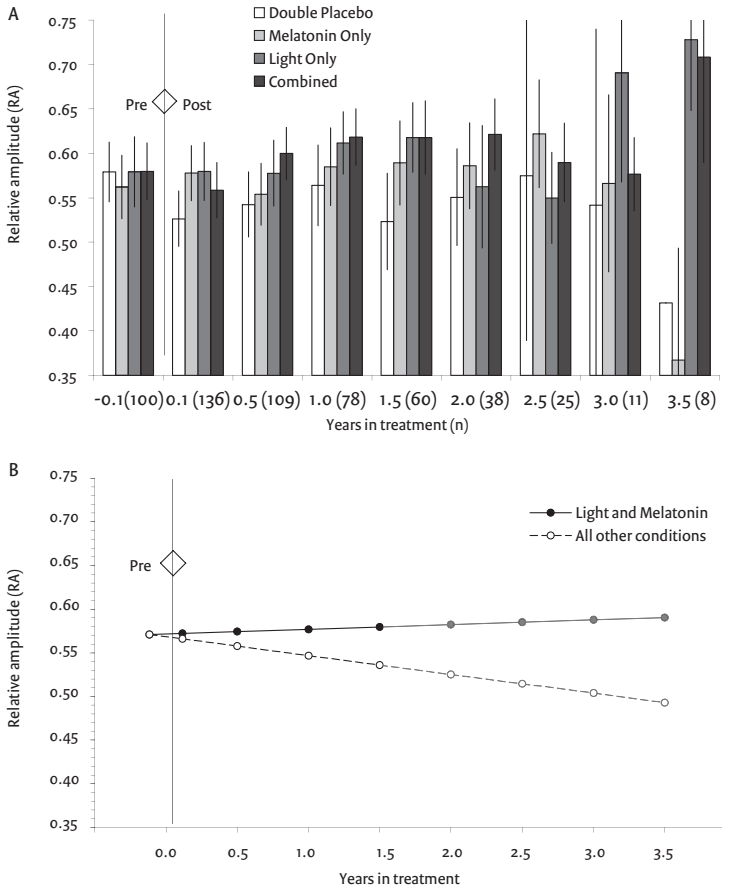


Figure 4.

Relative amplitude group averages per observation and graphical representation of regression parameters. (**A**): RA ratings (mean ± s.e.m.) four each group per follow-up assessment. Note the decline in number of observations (n, given between brackets on the horizontal axis) over the study period of three and a half years. Since the values obtained after 2 years of treatment are based on only a very limited number of subjects still included, they should be regarded of little value – as also indicated by the large standard errors. Note that the double placebo-group has a lower RA than the combined treatment group at all observations after the start of the treatments. Moreover, for all observations from 0.5 year to 2 years of follow-up, the combined treatment group maintains a higher RA than the groups receiving only light or only melatonin.

(**B**): Graphical representation of the most simple acceptable regression model for the 24-hour activity rhythm amplitude as assessed by RA. Given that the majority of data were collected within the first one a half year of treatment, the estimated effects after this period should be interpreted with caution, as indicated by the shading of the lines. Light and/or melatonin treatment commenced at time zero. Treatment started 6 weeks after the first assessment and 6 weeks prior to the second. As compared to any of the other conditions (combined in the dotted line, open circles), combined treatment (solid line, filled circles) increasingly enhanced RA over the years in treatment. The decline in the other line representing the other conditions is only due to the subjects that dropped out of the study due to nursing home placement or death: there was no uniform change over time in the other subjects. The curves show the weighed averages for subjects that dropped out of the study due to nursing home placement or death (contributing 49% of the data) and for subjects that provided assessments as long as their facility participated (51% of the data). The multilevel regression equation shown is: $RA_{ijk} = ß_{oijk} + ß_1$*Time*$DropoutPattern1_{ijk} + ß_2$*Time*Light*$Melatonin_{ijk}$ for each observation *i* in subject *j* of facility *k*. Effect coefficients are given in the text and in table 2.

Table 3. Evaluation of suspected adverse reactions suggested by previous studies on bright light or melatonin treatment. Throughout the study, caregivers provided 687 16-item ratings on a 4-point scale (0=absent, 1=probably absent, 2=probably present, 3=present). Note that light treatment significantly lowered the ratings on irritability, dizziness, headache, obstipation and inability to sleep, and melatonin the ratings on obstipation.

	Pre	Double placebo	Melatonin only	Light only	Melatonin +Light	Melatonin	Light
Dizziness	0.94 ± 0.10	0.89 ± 0.11	0.73 ± 0.09	0.44 ± 0.07	0.56 ± 0.08		****
Drowsiness	0.98 ± 0.11	0.97 ± 0.11	1.12 ± 0.11	0.93 ± 0.09	0.94 ± 0.11		
Eye complaints	0.86 ± 0.11	0.65 ± 0.09	0.74 ± 0.10	0.52 ± 0.07	0.65 ± 0.09		
Feebleness	0.69 ± 0.10	0.52 ± 0.09	0.73 ± 0.10	0.30 ± 0.06	0.43 ± 0.08		
Headache	0.75 ± 0.10	0.60 ± 0.08	0.86 ± 0.09	0.52 ± 0.07	0.55 ± 0.09		*
Hunger	0.38 ± 0.08	0.49 ± 0.08	0.32 ± 0.07	0.22 ± 0.05	0.22 ± 0.06		
Hyperactivity	0.26 ± 0.07	0.50 ± 0.09	0.34 ± 0.07	0.25 ± 0.05	0.16 ± 0.05		
Inabity to sleep	0.63 ± 0.09	0.94 ± 0.11	0.75 ± 0.09	0.20 ± 0.05	0.32 ± 0.07		****
Irritability	1.07 ± 0.12	1.29 ± 0.11	1.00 ± 0.10	0.93 ± 0.09	0.57 ± 0.09		**
Nausea	0.36 ± 0.08	0.40 ± 0.07	0.40 ± 0.07	0.27 ± 0.06	0.27 ± 0.07		
Obstipation	0.84 ± 0.10	0.88 ± 0.10	0.67 ± 0.09	0.46 ± 0.07	0.23 ± 0.06	*	**
Pins and needles	0.24 ± 0.06	0.46 ± 0.07	0.23 ± 0.05	0.09 ± 0.03	0.19 ± 0.06		
Stomach ache	0.23 ± 0.06	0.26 ± 0.05	0.31 ± 0.06	0.21 ± 0.05	0.11 ± 0.04		
Sweating	0.37 ± 0.08	0.48 ± 0.08	0.41 ± 0.08	0.26 ± 0.06	0.18 ± 0.05		
Trembling hands	0.37 ± 0.08	0.45 ± 0.08	0.56 ± 0.10	0.22 ± 0.05	0.39 ± 0.08		
Other complaints	0.48 ± 0.10	0.30 ± 0.08	0.41 ± 0.09	0.29 ± 0.07	0.28 ± 0.07		

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Regarding *sleep structure*, the combination of light and melatonin treatment interacted to reduce the average duration of individual brief nocturnal awakenings by 0.5±0.2 minutes (12%) per year (CI 0.21-0.85, p=0.01) , as compared to an unchanging duration of 4.3±0.2 minutes for assessments made under only one or no active treatments. ApoE-ε4 carriers (n=24) had a 0.8±0.4 minute (20%) longer average duration of intermittent nocturnal awakenings (CI 0.1-1.61, p=0.03). Melatonin treatment increased the average duration of uninterrupted periods of sleep by 5.8±2.4 minutes (25%) (CI 1-11 min, p=0.02) as compared to an unchanging duration of 24±2 minutes for assessments made under only one or no active treatments. Concerning the *24-hour sleep-wake pattern*, an important variable is how much activity disturbs the most restful five-hour period of the average activity profile. As compared to assessments made under only one or no active treatments, which remained unchanged at 12±0.6 minutes per hour, combined treatment reduced this activity by 1±0.4 minutes or 9% per year (CI 0.3-1.8, p=0.01). Because daytime activity levels were not affected by treatment, the reduction in nocturnal activity resulted in an increase of the 24-hour activity rhythm amplitude (RA). RA thus reflected nocturnal restlessness relative to the daytime activity level. As shown in table 2 and

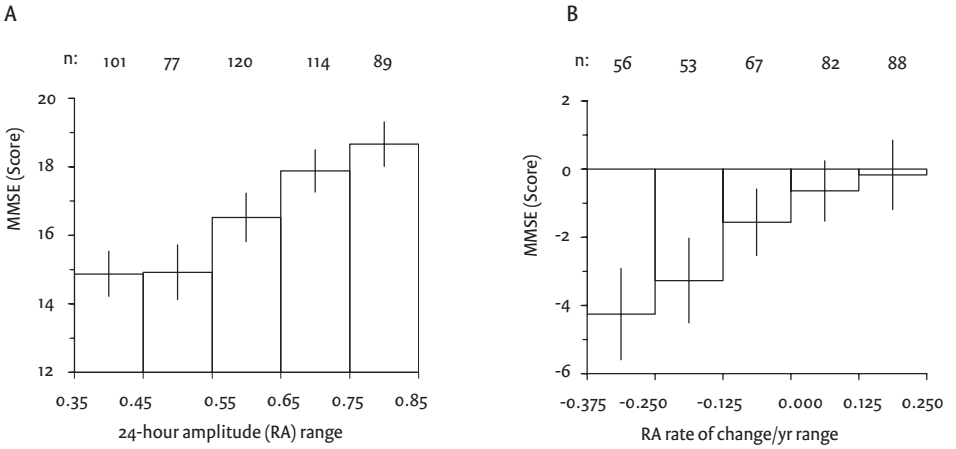


Figure 5.
Predictive value of the amplitude of the 24-hour pattern of sleep and wakefulness (RA) for cognitive performance (MMSE). (**A**): Mean±s.e.m. MMSE for five ranges of RA, from low to high. The middle three bins have equidistant range limits and cover about the center 60% of the available paired observations; the left and right bins extend to as low as the overall minimum RA value (0.08) and as high as the overall maximum RA value (0.98). The row of numbers (n) at the top of the 5a. indicates the number of available paired observations contributing to each bin. Note that the MMSE increases with increasing RA.
(**B**): Mean±s.e.m. annual rate of change on the MMSE for five ranges of annual rates of chance of RA. The middle three bins have equidistant range limits and cover about the center 60% of the available paired rate of change observations; the left and right bins extend to as low as the overall minimum RA-rate of change value (-2.02) and as high as the overall maximum RA-rate of change value (1.25). The row of numbers (n) at the top of the 5b. indicates the number of available paired observations contributing to each bin. Note that the more RA worsens, the stronger the decline in MMSE is, and that the decline in MMSE is limited for intervals where RA improves rather than worsens (rightmost two bins).Table 2. Regression effect estimates ± s.e.m. Outcome variables are listed in the left column. The second column gives the regression intercepts. Columns three to five show, respectively, the rate of change per year and the difference in level and rate of change for those subjects that left the study due to death or nursing home placement. The next 3 columns show the significant ($p<0.05$) treatment effects for Light, Melatonin and their interaction ('Combined'). The rightmost 3 columns show the treatment by time effects, given as rate of change per year. Blank cells indicate absence of significant (additional) effects. L5 = most restful five-hour period of the average 24-hour pattern, RA = relative amplitude, MMSE = mini mental state examination.

figure 4, the increase in RA was 0.03±0.01 or 5% per year (CI 0.01-0.05, p=0.01) when combined treatment was received as compared to the regression estimate of an unchanging 0.58±0.02 for assessments made under only one or no active treatments, decreasing over time only in those participants that dropped out of the study due to nursing home placement or death, as discussed below.

Missing data and effect-modulation by diagnosis

As mentioned, special attention was given to the fact that- especially after 1.5 years - many cases were lost from follow-up. First, we verified that treatment effects obtained from analyses on the complete 3.5 year dataset were still present in a reduced dataset including only the first 1.5 year of follow-up. As compared to the treatment effect estimates based on all available data, only marginal changes occurred if the estimates were derived on only the first 1.5 years of follow-up. In fact, treatment effect sizes increased by 14% on average if based on the first 1.5 years as compared to when they were based on the full dataset. Only one treatment effect - the effect of melatonin on sleep onset latency - lost significance, yet a trend remained ($p<0.06$). Thus, the effects reported are robust and cannot be attributed to confounding by dropout according to this sensitivity analysis.

The second approach was to code missing data due to (1) death or nursing home placement, or (2) non-compliance or insufficient communication abilities, in two dummy variables to allow for inclusion in the regression analysis according to a pattern mixture model approach. These analyses revealed that none of the treatment effects were modified by missing data patterns, even though subjects that dropped out of the study due to nursing home placement or death had a markedly worse and faster deteriorating MMSE and sleep-wake structure. The MMSE results have been mentioned already above. Concerning the sleep-wake structure, in these dropout subjects, RA declined by −0.04±0.01 or 8% per year (p=0.0001) relative to the regression equation intercept of 0.5±0.02. Their interdaily stability (IS, the normalised similarity between all recorded 24-hour periods, see methods) was −0.07±0.03 or 10% lower (p=0.01) and, moreover, declined by −0.05±0.01 or −7% per year (p=0.0001) relative to the regression equation intercept of 0.67±0.02. Their intradaily variability (IV, the fragmentation of periods of rest and activity, see methods) was 0.10±0.05 or 13% higher (p=0.03) and, moreover, increased by 0.07±0.02 or 10% per year (p=0.0005) relative to the regression equation intercept of 0.74±0.03. None of the treatment effects was modulated by the dummy-coded presence or absence of the diagnosis probable Alzheimer's disease.

Relationship between sleep and cognitive performance

The relationship between sleep and cognitive performance was investigated in three ways. Firstly, are cognitive performance ratings related to simultaneously obtained sleep parameters? Secondly, can the rate of cognitive decline be predicted from simultaneously occurring alterations in sleep? Thirdly, do treatment-induced changes in sleep contribute treatment effects on cognitive performance?

Firstly, a strong circadian component in the 24-hour pattern of sleep and wakefulness showed predictive value for cognitive performance as indicated by the parameters RA (p=0.0003), IS (p=0.0004), and IV (p=0.02). The strongest relationship with the MMSE was found for RA, and is shown in figure 5. A one point higher RA predicted a 4.6±1.3 points higher MMSE. This suggests that the range covered by RA observations (0.08-0.98) accounted for a range of 4.1 MMSE points. A one point higher IS predicted a 3.9±1.1 points higher MMSE, suggesting that the range of IS observations obtained (0.10-0.96) accounted for a range of 3.3 points on the MMSE. A one point higher IV predicted a 1.6±0.7 points lower MMSE, suggesting the range of IV observations (0.25-1.92) accounted for a 2.6 points MMSE range. The only within-sleep parameter associated with cognitive performance was sleep duration. Subjects with a long sleep duration had a lower MMSE (-0.42±0.14 MMSE points per hour, p=0.002).

Secondly, and in agreement with the above associations, attenuation of the circadian component in the 24-hour sleep-wake cycle also showed the strongest predictive value for an accelerated cognitive decline, as indicated by the annual rate of change in RA (p=0.003), IS (p=0.006), IV (p=0.008), and L5 (p=0.02). Once more, the strongest relationship was between the rates of change in MMSE and in RA, as shown in figure 5. Of the within-sleep parameters, only the rate of worsening sleep onset latency showed predictive value for an accelerated MMSE decline (p=0.03). Of note, the treatment-induced increase in sleep duration showed no predictive value for the MMSE decline, indicating that the negative association of sleep duration with MMSE reported above is strictly between-subject.

Thirdly, multilevel mediation analysis[31] showed a contribution of combined treatment-induced improvement in the 24-hour sleep-wake rhythm amplitude (RA) to the attenuation of cognitive decline (0.2±0.1 MMSE points per year, p=0.04).

Adverse effects evaluation

Caregivers provided a total of 694 adverse effects scale ratings, of which 571 follow-up assessments, from 182 subjects. Table 3 gives an overview of their average ratings. Items with the highest overall ratings were drowsiness and irritability, items with the least overall ratings stomach ache and pins and needles. Of note, none of the possible adverse effects as suggested in previous studies on light and melatonin worsened by either light or melatonin treatment or their interaction. On the contrary, as compared to the pre-treatment and placebo-condition assessments, light treatment significantly lowered the ratings on irritability, dizziness, headache, obstipation and inability to sleep. Melatonin lowered the ratings on obstipation. Concerning severe adverse events, none were reported by the patients' physicians. In the single otherwise reported case, the daughter of a patient – a 90 year old woman diagnosed with probable Lewy body dementia – suspected her mother's increase in restlessness and falls to be related to the treatment and requested discontinuation. This patient had been assigned to the double placebo condition.

Discussion

The results indicate that sleep and cognitive performance of the heterogeneous group of elderly residents in group care facilities benefit from daily supplementation with light and melatonin. Although the effect on cognition (0.9 MMSE points for light treatment) may seem marginal, it should be noted that it is comparable to the effect that can be accomplished in a younger, less affected and more homogeneous group of Alzheimer patients by means of acetylcholinesterase inhibitors, e.g. donepezil.[7] Whereas the effect of light on cognition appeared immediately, melatonin effects only surfaced after at least a year of treatment, and onwards (figure 3). Due to the limited number of subjects included in the later part of the study, it would be safest to limit the conclusions to the average follow-up period of fifteen months. The effects on sleep were in general most pronounced with combined light and melatonin treatment, and often increased over time, which may account for equivocal findings in short-term studies.[33]

Light ameliorated cognitive deficits, but did not decelerate its progressive worsening, resembling the effect that can be accomplished in a younger, less affected and more homogeneous group of Alzheimer patients by means of acetylcholinesterase inhibitors, e.g. donepezil.[7] Light treatment thus requires only a limited treatment duration before maximal effects are obtained.

With regards to melatonin effects, Singer et al.[34] reported nonsignificant trends for increased nocturnal total sleep time and decreased wake after sleep onset after two months of melatonin supplementation in Alzheimer's patients. In another study, Serfaty et al.[35] found no effect on sleep of two weeks of melatonin supplementation on demented elderly, and suggested that possible benefits of melatonin might surface only following longer periods of administration. Indeed, in our study, the prolonged duration of treatment and follow-up allowed us to demonstrate such slowly developing effects, that were moreover dependent on simultaneous light treatment.

A novel finding in our study was moreover that the *combination* of melatonin with light treatment appeared essential to induce an increase in the amplitude of the sleep-wake rhythm, and to induce improvements that developed over time in the mean duration of intermittent awakenings and in nocturnal restlessness. Since light and melatonin are regarded to be the primary Zeitgebers acting on the suprachiasmatic nuclei, the interaction effects suggest that a strong and bimodal (light during the day and melatonin during the night) input is most optimal to attain improved function in demented elderly subjects. Although the decreased level of melatonin at high age has been coined to be involved in age-related sleep problems (the "melatonin deficiency syndrome"), no evidence for such relation could be found[36]. Our

findings suggest that it could be worthwhile to investigate whether an interactive effect of low levels of melatonin with low levels of daytime light exposure could have a stronger relation to age-related sleep problems.

We hypothesize improved SCN function to have mediated the increase in the circadian amplitude of the sleep-wake rhythm. This increase appeared to have clinical relevance. First, the increased circadian amplitude mainly resulted from the decrease in the nocturnal activity by 9% per year. Since nocturnal restlessness is, as noted before, a decisive factor in caregiver burden and institutionalisation[4], studies in subjects still living at home are warranted. Second, there was a strong relation between the circadian amplitude of the sleep-wake rhythm and cognitive performance.

Only a limited number of previous studies have related ApoE genotype to disturbed sleep. ApoE-ε4 has been associated with long daytime naps[37] and an attenuated rate of deterioration of sleep efficiency.[19] A questionnaire study found that sleep disorders appeared more frequently in ApoE-ε3/4.[18] Our study adds to these limited data by showing ApoE genotype-related differences in disturbed sleep and in treatment efficacy. It should be noted that we succeeded to determine ApoE genotype from saliva in only 103 subjects – this limitation in combination with the low proportion of ApoE-ε2 and -ε4 carriers argue for a careful interpretation of our results, to be confirmed in larger samples. ApoE-ε4 carriers showed a more disturbed pattern of sleep: the duration of nocturnal awakenings increased by 20% for each additional ε4 allele. This effect was maintained in the verification analysis on the dataset limited to a follow-up duration of 1.5 year. Together with the previous report of Asada et al.[37], this indicates that ApoE-ε4 carriers have longer periods of sleep during the day and longer periods of wakefulness during the night, suggesting a more severely affected circadian organization. No previous studies investigated the relation of ApoE-ε2 to sleep. Our data suggested that the presence of an ApoE-ε2 allele was associated with an improved response of sleep duration on combined treatment. This effect was time-dependent, i.e. developed slowly. Likely due to a lack of statistical power for slowly developing effects, the modulating effect of the ApoE-ε2 could not be verified in the analyses limited to 1.5 years of follow-up. Still, the findings add sleep problems and possibly also the response to sleep-improving treatment to the overall less favourable prognosis for ApoE-ε4 carriers and more favourable prognosis for ApoE-ε2 carriers.[38, 39]

Two limitations of the present study should be discussed. First, the study was performed in a somewhat heterogeneous group of mostly demented elderly residents of group care facilities. Although the majority of the participants were diagnosed with probable Alzheimer's disease, the study thus did not focus on a strictly defined single nosological entity. However, the sample is representative for elderly residents of group care facilities, and results can be generalized at least to this strongly growing population, if not also to Alzheimer's disease, since the effects reported were not modified by this diagnosis. Second, one could argue that the increasing number of subjects lost to follow-up assessment was another limitation of the present study. The drop-out was primarily due to logistic limitations, i.e. discontinuation of facilities, and secondarily related to the very nature of the population under study, which is at high risk of death and transfer to a nursing home. We verified that the treatment effects were not modulated by dropout pattern and moreover turned out to be robust in a sensitivity analysis limiting the dataset to the first 1.5 years of follow-up only. Thus, the results cannot easily be attributed to a confounding effect of the large number of dropouts.

In agreement with experimental studies1 that have shown a contribution of sleep to daytime cognitive performance, we found a considerable co-variation of MMSE with sleep in elderly residents of group care facilities, the most pronounced being with the parameters that quantify the 24-hour sleep-wake cycle amplitude. In addition to their clinical importance, our findings provide proof of

principle for the idea that application of the appropriate stimuli can improve brain function even in elderly residents of group care facilities, many of which suffer from various neurodegenerative diseases. Within the limitations set by their age and /or disease, even relatively ordinary measures such as the application of light, melatonin, and possibly other regimens that enhance the stability of the sleep-wake rhythm, help to ameliorate disturbances of sleep and cognition.

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References

1. Walker MP, Stickgold R. Sleep-dependent learning and memory consolidation. Neuron 2004;44(1):121-33.

2. Fekete M, van Ree JM, Niesink RJ, de Wied D. Disrupting circadian rhythms in rats induces retrograde amnesia. Physiology & Behavior 1985;34(6):883-887.

3. Cho K. Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. Nature Neuroscience 2001;4(6):567-8.

4. Pollak CP, Perlick D. Sleep problems and institutionalization of the elderly. Journal of Geriatric Psychiatry & Neurology 1991;4(4):204-10.

5. Swaab DF, Fliers E, Partiman TS. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Research 1985;342(1):37-44.

6. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state': a practical method for grading the cognitive state of patients for the clinician. Journal of Psychiatric Research 1975;12:189-198.

7. Courtney C, Farrell D, Gray R, et al. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. Lancet 2004;363(9427):2105-15.

8. Moore RY. The organization of the human timing system. In: Swaab DF, Hofman MA, Mirmiran M, Ravid R, Van Leeuwen FW, eds. Progress in Brain Research. Amsterdam: Elsevier, 1992: 101-15.

9. Liu RY, Zhou JN, Hoogendijk WJG, et al. Decreased vasopressin gene expression in the biological clock of Alzheimer's disease patients with and without depression. Journal of Neuropathology & Experimental Neurology 2000;59(4):314-22.

10. Ingram CD, Ciobanu R, Coculescu IL, Tanasescu R, Coculescu M, Mihai R. Vasopressin neurotransmission and the control of circadian rhythms in the suprachiasmatic nucleus. Progress in Brain Research 1998;119:351-64.

11. Bliwise DL. Sleep in normal aging and dementia. Sleep 1993;16(1):40-81.

12. Witting W, Mirmiran M, Bos NP, Swaab DF. Effect of light intensity on diurnal sleep-wake distribution in young and old rats. Brain Research Bulletin 1993;30(1-2):157-62.

13. Lucassen PJ, Hofman MA, Swaab DF. Increased light intensity prevents the age related loss of vasopressin-expressing neurons in the rat suprachiasmatic nucleus. Brain Research 1995;693(1-2):261-266.

14. Van Someren EJW, Hagebeuk EEO, Lijzenga C, et al. Circadian rest-activity rhythm disturbances in Alzheimer's disease. Biological Psychiatry 1996;40:259-70.

15. Van Someren EJW, Kessler A, Mirmiran M, Swaab DF. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biological Psychiatry 1997;41:955-63.

16. Muzur A, Pace-Schott EF, Hobson JA. The prefrontal cortex in sleep. Trends In Cognitive Sciences 2002;6(11):475-81.

17. Moe KE, Vitiello MV, Larsen LH, Prinz PN. Sleep/wake patterns in Alzheimer's disease: relationships with cognition and function. Journal of Sleep Research 1995;4:15-20.

18. Cacabelos R, Rodrígues B, Carrera C, et al. APOE-related frequency of cognitive and noncognitive symptoms in dementia. Methods and Findings in Experimental Clinical Pharmacology 1996;18(10):693-706.

19. Yesavage JA, Friedman L, Kraemer H, et al. Sleep/wake disruption in Alzheimer's disease: APOE status and longitudinal course. Journal of Geriatric Psychiatry & Neurology 2004;17(1):20-4.

20. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington, DC: American Psychiatric Press, 1994.

21. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34(7):939-44.

22. McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB):

report of the consortium on DLB international workshop. Neurology 1996;47(5):1113-1124.

23. Sloane PD, Mathew LJ. The Therapeutic Environment Screening Scale. American Journal of Alzheimer's Care and Related Disorders and Research 1990;5:22-6.

24. Slaughter S, Calkins M, Eliasziw M, Reimer M. Measuring physical and social environments in nursing homes for people with middle- to late-stage dementia. Journal of the American Geriatrics Society 2006;54(9):1436-41.

25. Middleton B, Stone BM, Arendt J. Human circadian phase in 12:12 h, 200: <8 lux and 1000: <8 lux light-dark cycles, without scheduled sleep or activity. Neuroscience Letters 2002;329(1):41-4.

26. Kushida CA, Chang A, Gadkary C, Guilleminault C, Carrillo O, Dement WC. Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. Sleep Medicine 2001;2(5):389-396.

27. Van Someren EJW, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiology International 1999;16(4):505-18.

28. Sjogren M, Hesse C, Basun H, et al. Tacrine and rate of progression in Alzheimer's disease--relation to ApoE allele genotype. Journal of Neural Transmission 2001;108(4):451-8.

29. Twisk JWR. Applied longitudinal data analysis for epidemiology. Cambridge: Cambridge University Press, 2003.

30. Siddiqui O, Ali MW. A comparison of the random-effects pattern mixture model with last-observation-carried-forward (LOCF) analysis in longitudinal clinical trials with dropouts. Journal of Biopharmaceutical Statistics 1998;8(4 (Nov)):545-63.

31. Krull JL, MacKinnon DP. Multilevel modeling of individual and group level mediated effects. Multivariate Behavioral Research 2001;36(2):249-277.

32. Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. Sleep 2003;26(3):342-92.

33. Forbes D, Morgan D, Bangma J, Peacock S, Pelletier N, Adamson J. Light Therapy for Managing Sleep, Behaviour, and Mood Disturbances in Dementia. Cochrane Database Systematic Reviews 2004;2: CD003946.

34. Singer C, Tractenberg RE, Kaye J, et al. A multicenter, placebo-controlled trial of melatonin for sleep disturbance in Alzheimer's disease. Sleep 2003;26(7):893-901.

35. Serfaty M, Kennell-Webb S, Warner J, Blizard R, Raven P. Double blind randomised placebo controlled trial of low dose melatonin for sleep disorders in dementia. International Journal of Geriatric Psychiatry 2002;17(12):1120-7.

36. Baskett JJ, Wood PC, Broad JB, Duncan JR, English J, Arendt J. Melatonin in older people with age-related sleep maintenance problems: a comparison with age matched normal sleepers. Sleep 2001;24(4):418-24.

37. Asada T, Motonaga T, Yamagata Z, Uno M, Takahashi K. Associations between retrospectively recalled napping behavior and later development of Alzheimer's disease: association with APOE genotypes. Sleep 2000;23(5):629-34.

38. Cacabelos R. Pharmacogenomics and therapeutic prospects in Alzheimer's disease. Expert Opinion on Pharmacotherapy 2005;6(12):1967-87.

39. Martins CA, Oulhaj A, de Jager CA, Williams JH. APOE alleles predict the rate of cognitive decline in Alzheimer disease: a nonlinear model. Neurology 2005;65(12):1888-93.

Chapter 8

Prevention of non-cognitive symptoms in elderly residents of group care facilities by long-term bright-light and melatonin treatment

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Abstract

Background: Depression, behavioural disturbances, and limitations of activities of daily living commonly burden demented elderly and their caregivers. The reported association with sleep and circadian rhythm disturbances argue for a chronobiological approach of the long-term management of these symptoms. We here report on the non-cognitive outcomes of the first long-term double-blind placebo-controlled follow-up study to chronically apply the combination of the two major synchronizers of the circadian timing system, bright light and melatonin.
Methods: Mostly demented elderly (n=189) of 12 group care facilities were randomly assigned to long-term daily treatment with whole-day bright (±1000 lux) or dim (±300 lux) light and evening melatonin (2.5 mg) or placebo for 15±1 months (mean±s.e.m.) on average and up to 3.5 years for a limited number of participants. Non-cognitive symptoms were repeatedly assessed using standardized scales. Statistical analyses consisted of mixed effects regression analyses.
Results: Light ameliorated depressive symptoms by 19% (p=0.02) and attenuated the increase over time in limitations of activities of daily living by 53% (p=0.003). Melatonin ameliorated the severity and distress of psychiatric symptoms in subjects about to drop out of the study due to nursing home placement or death by 26% per year (p=0.04) and 45% per year (p=0.003) respectively. However, melatonin adversely affected affect and withdrawn behaviour (p≤0.02). These adverse effects were partly counteracted if melatonin was given in combination with light. The combined treatment furthermore attenuated aggressive behaviour by 9% (p=0.01). Especially with light treatment, previously suggested adverse effects improved rather than worsened.
Conclusions: Light and melatonin are valuable in the long-term management of non-cognitive symptoms in dementia. Light ameliorates the progressive worsening of depressive symptoms. Melatonin ameliorates behavioural disturbances but is recommended for chronic use in dementia only in combination with light, to counteract its adverse effect on mood.

Introduction

The worldwide prevalence of people suffering from dementia is 24.3 million, and is expected to double every 20 years in the next decades.[1] To date no therapy is available that can stop the disease process. We are still facing a devastating progressive illness and only a slight attenuation of the cognitive decline can be attained by pharmacological treatment.[2] In addition to the cognitive decline, patients, caregivers and clinicians are confronted with disturbances of mood and behaviour. The prevalence of these non-cognitive mental disturbances is higher in demented elderly than in age-matched non-demented elderly.[cf. 3,4] The prevalence of depression in Alzheimer patients is estimated to be 30-50%.[cf. 3] Depression is correlated with reduced quality of life,[5] increased caregiver burden[6] and higher mortality rates.[7] Furthermore, depression is an important independent risk factor for early institutionalization.[8] Over 50% of the demented outpatients also show behavioural disturbances.[9] Like depression, these put a high burden on caregivers and are another important reason for institutionalization.[10]

Treatment of both depression and behavioural disturbances usually consists of the prescription of psychotropic drugs. Regarding neuropsychiatric symptoms there is only modest effectiveness of antipsychotics, while they have the disadvantage of adverse effects, especially in the elderly.cf.[11] For the pharmacological treatment of depression in the elderly either classical tricyclic antidepressants (TCA) or selective serotonin reuptake inhibitors (SSRI) are prescribed, with approximately identical antidepressant effect.cf.[12] The moderate effectiveness and the adverse effects of the available drugs create the opportunity for other treatment strategies.

A variety of studies have suggested a role of the circadian pacemaker in depression. Typical findings in depressed patients are that they feel worst in the morning and suffer from early morning awakenings.[13,14] Another consistent finding is a decrease in the amplitude of the body temperature in depressed patients.[13,14] Furthermore, the successful treatment of seasonal affective disorder with bright light,[15] the primary circadian "Zeitgeber", has led to the hypothesis that the antidepressant effect of bright light acts via the retinohypothalamic tract on the circadian pacemaker, i.e. the suprachiasmatic nucleus. In post-mortem studies further evidence was found for a disturbance in the suprachiasmatic nucleus in depression as well as in dementia.[16-18] In depression, the number of vasopressin immunoreactive neurons was increased along with a decrease in the expression of vasopressin mRNA, suggesting that synthesis and transport of vasopressin are reduced.[17] In demented elderly, the expression of vasopressin mRNA was also lower than the level found in control subjects.[18] The circadian timing system (CTS) is highly sensitive to both environmental bright light and the pineal hormone melatonin.[19] In the absence of their synchronizing effects, circadian rhythms start to free run. In demented elderly, this daily synchronization is attenuated because subjects seldom expose themselves to bright light and their nocturnal melatonin production is reduced.[20,21]

Therapeutic effects of bright light and melatonin on mood and behavioural disturbances in demented elderly have indeed been suggested.[22,23] However, these studies were of limited duration and sample size, and have also been criticized for not being able to meet the quality criteria that are required to draw reliable conclusions.[24] No previous human studies have applied long-term combined stimulation of the CTS with daily bright light and melatonin. We report here on the results of the first multicenter double-blind placebo-controlled randomized follow-up study (ISRCTN registry number 93133646) to evaluate the effects of long-term (up to 3.5 years) daily supplementation of bright light and/or melatonin on mood and behavioural disturbances in mostly demented elderly residents of group care facilities. Importantly, we designed and verified a novel procedure to accomplish a true placebo light condition.

Methods

Subjects

Participants were 189 residents of 12 different Dutch homes for the elderly (170 women, 19 men, mean age 85.8±0.4 mean±s.e.m. years) living in assisted care facilities, where subjects have their own apartment where they sleep and retreat, but spend most of the daytime in a common living room. The Medical Ethics Committees of Hospital "De Gelderse Vallei", Ede and the VU University Medical Center, Amsterdam, The Netherlands, approved the study, and subjects only participated after informed consent of their relatives.

The clinical diagnosis of dementia was made according to the DSM-IV criteria for dementia and dementia subtypes.[25] NINCDS-ADRDA criteria were used for the clinical diagnosis of probable Alzheimer's disease.[26] Of the 189 subjects, 120 (63%) met the NINCDS-ADRDA criteria for probable Alzheimer's disease, 20 (11%) met DSM-IV criteria for Vascular dementia, 24 (13%) subjects met criteria for other types of dementia, including Dementia due to Multiple Etiologies (9 cases), Frontal type dementia (3 cases), Lewy body dementia (2 cases), Parkinson's disease (2 cases), Wernicke-Korsakoff (1 case) and Dementia not otherwise specified (7 cases). Seventeen subjects (8%) did not meet the criteria for dementia, but stayed in the group care facility for various medical or psychosocial reasons. In 8 subjects, data on medical history were insufficient to come to a reliable clinical diagnosis.

Treatment

Subjects were randomly assigned to double blind daily intake of melatonin (2.5

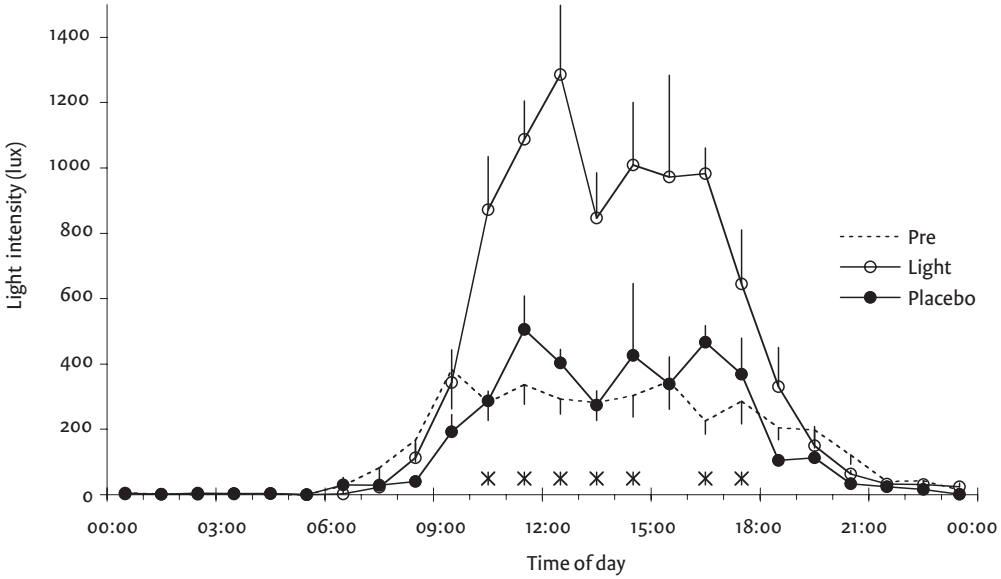


Figure 1
Average 24-hour light exposure profiles before (dashed line) and after installing the active (open circles) and placebo (closed circles) light conditions. Illumination levels were obtained at eye level in the direction of gaze, which was usually slightly downward or at best vertical. Note that such levels are considerably lower than horizontal assessments directed towards the light sources, but better represent light levels, as they can enter the eye. Assessments include occasional observations made if subjects were not actually present in the common living room where the lights were installed but in their own bedroom. Asterisks indicate the hours of significantly increased light intensity in the active condition ($p \leq 0.01$). None of the hours showed increased intensity in the placebo condition. Mean ± s.e.m. values were obtained from multilevel analysis of 3017 light measurements from 189 subjects in 12 facilities.

mg, Terafarm, Brielle, The Netherlands, n= 95) or placebo (n= 94), approximately 1 hour before bedtime. The twelve homes for the elderly were randomly assigned to active (6 facilities, n= 98) or placebo (6 facilities, n= 91) light exposure. Forty-nine subjects were assigned to light only, 46 to melatonin only, 49 to their combination and 45 to double placebo. The ratio of subjects assigned to the active melatonin condition within each facility was 0.50±0.02 (mean±s.e.m.). Successful randomization was verified by the lack of difference between subjects assigned to the active or placebo conditions at their initial assessment on any of the outcome variables obtained (average p=0.49).

Light exposure was manipulated by installing a large number of ceiling-mounted fixtures with Plexiglas diffusers containing an equal amount of Philips TLD 840 and 940 fluorescent tubes in the common living room. Lights were on daily between approximately 9:00 and 18:00 hr. We aimed at an exposure of ±1000 lux, measured before the eyes in gaze direction. Such daytime light intensity has in previous studies been confirmed to synchronize circadian rhythms in healthy subjects in temporal isolation[27] and to improve circadian activity rhythm disturbances in moderately to severely demented elderly.[28] Moreover, the latter study indicated that an exposure of ±1000 lux is both feasible and tolerated in most group care facilities, while higher intensities would take considerably more technical effort. In the placebo condition an equal amount of fixtures was installed, but these contained only half of the tubes, accommodated concealed band stop

filters and were installed at a larger distance from the eyes. The effectuated average light exposure measured at eye-level in the gaze direction is shown in figure 1. Caregivers were blind to the condition of their facility, as was verified by a lack of difference between 184 ratings obtained from 89 caregivers over the treatment period on an a illumination pleasantness visual analogue scale ($p=0.47$) and on the odds of a confirmative answer on the question whether they thought their facility had effective light ($p=0.62$).

Procedure

Subjects were followed for up to 3.5 years, on average 15 ± 1 month. In 129 subjects, assessments of neuropsychiatric symptoms started 6 weeks prior to the start of the treatment. Follow-up assessments were made 6 weeks after the start of the treatment, and subsequently every six months up to a maximum of 3.5 years. Another 60 subjects were enrolled in the study later: lights had already been installed before their admission to the care facilities. After enrolment, a subject's maximal follow-up period was primarily determined by the duration of participation of their facility. Due to logistical reasons including rebuilding, moving and staff limitations, participation of facilities varied between 3.5 years (four facilities); 3 years (one facility); 2.5 years (two facilities);); 2.5 years (two facilities); 1.5 year (two facilities) and 0.5 year (one facility). Secondarily, a major number of subjects were lost from follow-up assessment due to death or outplacement to a nursing home Figure 2 A. shows the cumulative frequency distribution of the percentage of subjects available for observation and the percentage lost due to retreat, nursing home placement or death. Figure 2 B. shows a flow chart of subjects included in the study.

On each occasion, neuropsychiatric symptoms and limitations of activities of daily living were determined by an assessment battery of validated scales (see below) including direct observations by a neuropsychologist and indirect observations by caregivers. Both the neuropsychologist and caregivers were blind to the randomized condition of the patient. For neuropsychological assessment, the patient was transferred from the care facility to a separate office.

Assessment scales

Two scales were administered by a neuropsychologist. The Cornell scale for depression in demented elderly (CSDD) is a 19-item scale specifically designed and validated for the rating of depressive symptoms in demented patients.[29,30] Higher ratings indicate more depressive symptoms. The scale is administered in two steps. First, the rater interviews the patient's caregiver on each of the items, and then briefly interviews the patient. In case of discrepancy, the rater interviews the caregiver to clarify the reason for disagreement. After this, the scale is scored on the basis of the rater's final judgement. The neuropsychologist also obtained the self-esteem of the patient using the 17 item Philadelphia Geriatric Centre Morale Scale (PGCMS) that includes items on agitation, the attitude towards one's aging, and dissatisfaction due to loneliness.[31]

Six scales were filled out by the daily caregivers. The Philadelphia Geriatric Centre Affect Rating Scale (PGCARS) is an observation scale for behavioural expressions of both negative and positive mood.[32] Withdrawn Behaviour was assessed with an 8-item subscale of the Multi Observational Scale for Elderly Subjects (MOSES).[33] The subscale contains items on social contacts and interest in everyday events. Higher scores indicate a more severe lack of social interaction. The Questionnaire format of the Neuropsychiatric Inventory (NPI-Q) was used to assess psychopathology.[34,35] For each of the 12 items, the caregiver gives a separate rating for the severity of and the resulting distress. The Cohen-Mansfield Agitation Inventory (CMAI) was used to rate how often a subject manifested 29 agitated behaviours.[36,37] Higher ratings on the CMAI indicate the presence of more frequent and more severe agitated behaviour. Limitations of activities of daily

living were rated on the nurse-informant adaptation[38]of the Katz scale.[39] Higher ratings on the NI-ADL indicate more limitations of activities of daily living, and item scores can sum up to a maximum of 58. Item-average scores are provided here, ranging form 0 to 4.8. Finally, caregivers rated 16 items on possible adverse effects suggested from previous studies on light or melatonin treatment on a 4-point scale (0=absent, 1=probably absent, 2=probably present, 3=present).

Statistical analysis

The data set consisted of a variable number of time points obtained from different individuals grouped in 12 homes for the elderly. In addition to the variable duration of participation of the homes for the elderly, and inherent to the study population, many subjects dropped out due to either passing away or nursing home placement. Incomplete data could furthermore be caused by non-compliance or non-attendance at some of the assessments. Another source of variability in the number of assessments per subject was introduced by allowing subjects to enter the study also if they were transferred to the care facility only after the study had already begun. Mixed effect regression analyses[40] were applied to account for the three-level nested structure of the dataset, i.e. a variable number of observations nested within subjects, and subjects grouped in 12 facilities. All analyses were performed with the MLwiN software (Institute of Education, London, UK). Melatonin, light and their interaction were dummy coded in three variables indicating the presence of active treatment at any observation. Both treatment-effects (i.e. independent of time) and time-by-treatment effects (i.e., treatment effects evolving slowly over time) were evaluated. In addition, the regression models allowed for inclusion of linear changes over time, and for modulation of level, time course and treatment effect by ApoE genotype and by missing data patterns. In the analyses, special attention was given to the fact that- especially after 1.5 years - many cases were lost from follow-up either due to noninformative reasons (discontinuation of participation of the facility) or to possibly informative causes. First, in order to obtain the most simple acceptable regression equation insensitive to a reduction in the follow-up time, we verified whether treatment effects obtained from analyses on the complete 3.5 year dataset were still present in a reduced dataset including only the first 1.5 year of follow-up. A second approach was to code missing data due to (1)

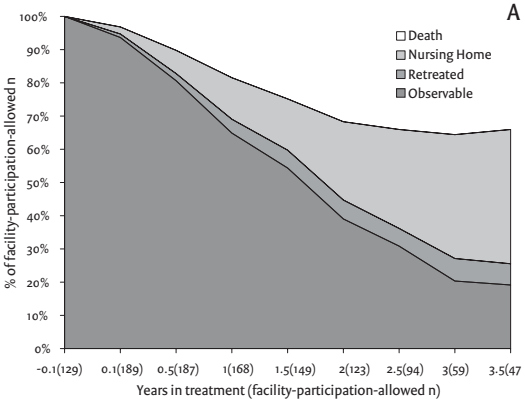
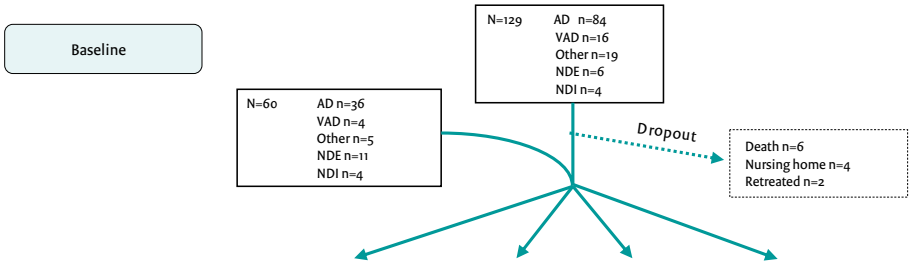


Figure 2

A. Cumulative frequency distribution of the percentage of subjects available for observation and the percentage lost due to retreat, nursing home placement or death. At each assessment point (horizontal axis) the percentage is expressed relative to the facility-participation allowed maximal number of subjects had everybody still been observable (given in brackets at the horizontal axis). Note that, of the observable cases, a percentage of assessments missed due to logistics, non-compliance or insufficient communication abilities (see text for numbers).
B. Flow chart of subjects included in the study. One hundred twenty nine subjects started with the pre-treatment assessment (AD = Alzheimer's disease, VAD = vascular dementia, other = other types of dementia, NDE = not demented, NDI = not diagnosed due to lack of data). Sixty subjects entered the study later (see text) and are included in the chart from post-treatment assessment 1 onwards, irrespective of the time relative to their facilities duration of participation. Reasons for dropout were death, nursing home placement, retreated informed consent or study end of the facility. The first post-treatment assessment 1 was 6 weeks after the start of the light and/or melatonin treatment, assessment 2 half a year after the start of light and/or melatonin and the subsequent assessments were done every next half year.

B



Post-treatment 1 6 wk	Light & Melatonin Total n=43	Light Total n=48	Melatonin Total n=46	Placebo Total n=40
	31 AD 6 VAD 3 Other 3 NDE 0 NDI	36 AD 5 VAD 6 Other 1 NDE 0 NDI	28 AD 3 VAD 6 Other 5 NDE 4 NDI	20 AD 4 VAD 7 Other 8 NDE 1 NDI

Post-treatment 2 6 months	N=150	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatonin	36		4	1	0	2
Light	44		2	2	0	0
Melatonin	39		2	3	1	1
Placebo	31		5	3	1	0

Post-treatment 3 1 year	N=109	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatonin	26		1	5	0	4
Light	34		0	3	0	7
Melatonin	27		7	3	0	2
Placebo	22		5	1	3	0

Post-treatment 4 1.5 years	N=81	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	21		1	1	0	3
Light	24		4	2	0	4
Melatonin	18		4	2	0	3
Placebo	18		2	0	1	1

Post-treatment 5 2 years	N=48	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	12		4	3	0	2
Light	17		3	2	0	2
Melatonin	9		3	2	0	4
Placebo	10		1	2	1	4

Post-treatment 6 2.5 years	N=29	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	9		2	0	0	1
Light	14		1	1	0	1
Melatonin	4		0	2	0	3
Placebo	2		2	1	0	5

Post-treatment 7 3 years	N=12	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	3		0	2	0	4
Light	5		0	2	0	7
Melatonin	2		0	0	0	2
Placebo	2		0	0	0	0

Post-treatment 8 3.5 years	N=9	Dropout	Death	Nursing home	Retreated	Study end
Light & Melatoni n	3		0	0	0	0
Light	3		1	0	0	1
Melatonin	2		0	0	0	0
Placebo	1		0	1	0	0

Table 1. Time and treatment effects on mood, behaviour and limitations of activities of daily living in mostly demented elderly residents of group care facilities. CSDD=Cornell scale for depression in dementia, PGCARS=Philadelphia Geriatric Center Affect Rating Scale, PGCMS=Philadelphia Geriatric Center Morale Scale, MOSES=Multidimensional Observation Scale for Elderly Subjects, NPI-Q=Neuropsychiatric Inventory-Questionnaire, CMAI=Cohen-Mansfield Agitation Inventory, NI-ADL=Nurse Informant version of the Katz Activites of Daily Living scale. Treatment effects were not modulated by time in treatment, except for the effect of light on the NI-ADL and the effect of melatonin on the NPI-Q; the latter only present in subjects that dropped out of the study due to outplacement to a nursing home, or death.

		Time effect (points/year)			Treatment effects		
Parameters	Intercept	Dropout	Time	Dropout*Time	Light	Melatonin	Light*Melatonin
Mood scales							
CSDD	7.7 ± 0.7		+2.1 ± 0.3	1.5 ± 0.4	-1.5 ± 0.6		
PGCARS							
positive	11.3 ± 0.4	-0.9 ± 0.3				-0.5 ± 0.2	
negative	5.9 ± 0.3	0.9 ± 0.3				0.8 ± 0.3	-1.0 ± 0.4
PGCMS	11.5 ± 0.3						
Behavioral scales							
MOSES	15.6 ± 0.9	4.4 ± 0.7	+1.1 ± 0.2			1.0 ± 0.4	
NPI-Q							
severity	4.9 ± 0.5			2.1 ± 0.4		-1.3 ±0.6/year*	
caregiver distress	4.6 ± 0.5			2.5 ± 0.5		-2.1 ±0.7/year*	
CMAI	44.7 ± 2.1		+1.3 ± 0.6	4.2 ± 1.0			-3.9 ± 1.5
Functional scale							
NI-ADL	1.39 ± 0.18	0.46 ± 0.14	0.28 ± 0.04	0.24 ± 0.05	-0.15 ± 0.05 /year		

*dropouts only

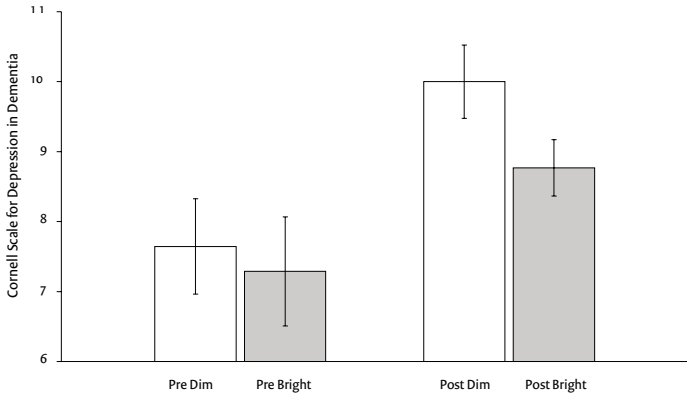


Figure 3
Ratings (mean ± s.e.m.) on the Cornell scale for depression in dementia (CSDD) in the active light (filled bars) and placebo light (open bars) conditions prior to and after the start of the treatment. CSDD scores increased significantly less in the active light treatment group (p=0.02, see text).

Table 2. Possible adverse effects suggested by previous studies on bright light or melatonin treatment. Throughout the study. caregivers provided 687 16-item ratings on a 4-point scale (0=absent. 1=probably absent. 2=probably present. 3=present). Note that light treatment significantly *lowered* the ratings on irritability. dizziness. headache. obstipation and inabity to sleep. and melatonin the ratings on obstipation.

	Pre	Double placebo	Melatonin only	Light only	Melatonin +Light	Melatonin	Light
Dizziness	0.94 ± 0.10	0.89 ± 0.11	0.73 ± 0.09	0.44 ± 0.07	0.56 ± 0.08		****
Drowsiness	0.98 ± 0.11	0.97 ± 0.11	1.12 ± 0.11	0.93 ± 0.09	0.94 ± 0.11		
Eye complaints	0.86 ± 0.11	0.65 ± 0.09	0.74 ± 0.10	0.52 ± 0.07	0.65 ± 0.09		
Feebleness	0.69 ± 0.10	0.52 ± 0.09	0.73 ± 0.10	0.30 ± 0.06	0.43 ± 0.08		
Headache	0.75 ± 0.10	0.60 ± 0.08	0.86 ± 0.09	0.52 ± 0.07	0.55 ± 0.09		*
Hunger	0.38 ± 0.08	0.49 ± 0.08	0.32 ± 0.07	0.22 ± 0.05	0.22 ± 0.06		
Hyperactivity	0.26 ± 0.07	0.50 ± 0.09	0.34 ± 0.07	0.25 ± 0.05	0.16 ± 0.05		
Inabity to sleep	0.63 ± 0.09	0.94 ± 0.11	0.75 ± 0.09	0.20 ± 0.05	0.32 ± 0.07		****
Irritability	1.07 ± 0.12	1.29 ± 0.11	1.00 ± 0.10	0.93 ± 0.09	0.57 ± 0.09		**
Nausea	0.36 ± 0.08	0.40 ± 0.07	0.40 ± 0.07	0.27 ± 0.06	0.27 ± 0.07		
Obstipation	0.84 ± 0.10	0.88 ± 0.10	0.67 ± 0.09	0.46 ± 0.07	0.23 ± 0.06	*	**
Pins and needles	0.24 ± 0.06	0.46 ± 0.07	0.23 ± 0.05	0.09 ± 0.03	0.19 ± 0.06		
Stomach ache	0.23 ± 0.06	0.26 ± 0.05	0.31 ± 0.06	0.21 ± 0.05	0.11 ± 0.04		
Sweating	0.37 ± 0.08	0.48 ± 0.08	0.41 ± 0.08	0.26 ± 0.06	0.18 ± 0.05		
Trembling hands	0.37 ± 0.08	0.45 ± 0.08	0.56 ± 0.10	0.22 ± 0.05	0.39 ± 0.08		
Other complaints	0.48 ± 0.10	0.30 ± 0.08	0.41 ± 0.09	0.29 ± 0.07	0.28 ± 0.07		

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

death or nursing home placement and (2) non-compliance or insufficient communicative abilities at any assessment occasion were considered to be informative and dummy coded (indicating presence of this condition for a subject at any point in time) to allow for evaluation of their possible effects in a pattern mixture model.[41] Possible effect-modulations by diagnosis were examined by including dummy coding of the Alzheimer's diagnosis in the regression models. The most simple acceptable regression equations were selected using the likelihood ratio chi-square test.

Results

Mood scales

Table 1 provides an overview of all time and treatment effects. The neuropsychologist obtained a total of 730 succesful CSDD depression evaluations, of which 606 follow-up assessments, from 187 subjects. Of the maximum number of possible observations (744), 2% was lost due to logistics. The grand mean was 8.8 ± 0.6 (mean ± s.e.m.) out of a maximum score of 38. CSDD ratings at baseline were 7.7±0.7 points and increased by 2.1±0.3 points per year and an additional 1.5 ± 0.4 points per year in the subjects that dropped out of the study due to transfer to a nursing home or death. Light treatment attenuated the increasing CSDD depression scores by 1.5±0.6 points or 19% (p=0.02) (see figure 3). The neuropsychologist furthermore

obtained a total of 604 PGCMS scores on the subjects' self-esteem, of which 492 follow-up assessments, from 172 subjects. Of the maximum number of observations (744), 18% failed due to non-compliance or insufficient communication abilities and 1% due to logistics. The grand mean was 11.5±0.3. Neither light nor melatonin significantly affected the PGCMS total score. However, a light by time interaction indicated an improvement of 0.15±0.07 or 3% (p=0.03) per year on the Lonely dissatisfaction subscale, which measures the dissatisfaction with the experienced interaction (not included in table 1).

Of the maximally possible number (744) of caregiver ratings on the PGCARS, MOSES, NPI-Q, CMAI and NI-ADL, 4% failed because caregivers felt unable to provide a rating, related to limitations of communication abilities or observability of the subjects, and 1% due to logistics. Caregivers provided a total of 699 PGCARS mood observations, of which 576 follow-up assessments, from 182 subjects. The grand mean of the positive mood subscale was 10.6±0.4 (out of a maximum of 15). Baseline ratings were 11.3±0.4 points and 0.9±0.3 points lower in subjects that dropped out of the study due to transfer to a nursing home or to death. Melatonin treatment lowered the positive mood rating by 0.5±0.2 or 5% (p=0.02). The grand mean of the negative mood subscale was 6.5±0.3 (out of a maximum of 15). Baseline ratings were 5.9±0.3, and 0.9±0.3 points higher in subjects that dropped out due to transfer to a nursing home or death. Melatonin treatment increased the negative mood rating by 0.8±0.3 or 14% (p<0.001). A light by melatonin interaction effect of -1.0±0.4 or 17% (p=0.02) indicated that the adverse effect of melatonin on positive and negative mood expressions was compensated for those subjects who received bright light in addition to melatonin. None of the treatment effects on mood was modulated by the dummy-coded presence or absence of the diagnosis Alzheimer's disease.

Behavioural scales

Caregivers provided a total of 701 MOSES-withdrawn behaviour subscale ratings, of which 577 follow-up assessments, from 182 subjects. The grand mean was 18.9±0.5 (out of a maximum of 34). Baseline ratings were 15.6±0.9 points and 4.4±0.7 higher in subjects that dropped out of the study due transfer to a nursing home or death. Withdrawn behaviour increased over time by 1.1±0.2 points per year. Melatonin treatment aggravated withdrawn behaviour by 1.0±0.4 or 7% (p=0.02).

A total of 706 NPI-Q ratings on psychopathology of were obtained from the caregivers, of which 581 follow-up assessments, from 183 subjects. The grand mean of the NPI-*severity* subscale was 5.2±0.5 out of a maximum of 36. Ratings were 4.9±0.5 at baseline and increased by 2.1±0.4 points per year only in subjects that dropped out of the study due to transfer to a nursing home or to death. Melatonin treatment attenuated the increase over time in these subjects by 1.3±0.6 per year (p=0.04), i.e. a 62% improvement of the slope (rate of change). The grand mean of the NPI-Q *caregiver distress* subscale was 5.0±0.5 out of a maximum of 60. Ratings were 4.7±0.5 at baseline and increased by 2.5±0.5 points per year only in subjects that dropped out of the study due to transfer to a nursing home or death. Melatonin treatment diminished the increase over time in these subjects by 2.1±0.7 per year (p=0.003), i.e. an 84% improvement of the slope (rate of change). Caregivers provided 708 CMAI ratings on agitated behaviour, of which 583 follow-up assessments, from 184 subjects. The grand mean was 45.81±2.0 out of a maximum of 203. Ratings were 44.7±2.1 at baseline and increased by 1.3±0.6 points per year and an additional 4.2±1.0 points per year in subjects who dropped out of the study due to transfer to a nursing home or death. Combined light and melatonin treatment attenuated the CMAI score by 3.9±1.5 or 9% (p=0.01). None of the treatment effects on behavior was modulated by the dummy-coded presence or absence of the diagnosis Alzheimer's disease.

Activities of daily living

Caregivers provided a total of 700 NI-ADL ratings, of which 575 follow-up assessments, from 181 subjects. The grand mean was 1.81±0.14 (item average), out of a maximum of 4.8. Baseline ratings were 1.39±0.18 and 0.46±0.14 higher in subjects that dropped out of the study due transfer to a nursing home or death. The limitations in ADL increased by 0.28±0.04/ per year and an additional 0.24±0.05 per year in the drop-out subjects. Light treatment attenuated the increase in ADL limitations by 0.15±0.05/year, i.e. a 53% less steep decline as compared to the baseline decrease of 0.28 per year (p=0.003).

Missing data and effect-modulation by diagnosis

As mentioned, special attention was given to the fact that- especially after 1.5 years - many cases were lost from follow-up. First, we verified that treatment effects obtained from analyses on the complete 3.5 year dataset were still present in a reduced dataset including only the first 1.5 year of follow-up. As compared to the treatment effect estimates based on all available data, only marginal changes occurred if the estimates were derived on only the first 1.5 years of follow-up. In fact, positive treatment effect size estimates increased by 8% on average if based on the first 1.5 years as compared to when they were based on the full dataset. Negative treatment effect sizes dropped by 9%, and the negative effect of melatonin on the MOSES lost significance and was present only as a trend (p=0.07). Thus, the effects reported are robust and cannot be attributed to confounding by dropout according to this sensitivity analysis.

The second approach was to code missing data due to (1) death or nursing home placement, or (2) non-compliance or insufficient communication abilities, in two dummy variables to allow for inclusion in the regression analysis according to a pattern mixture model approach. These analyses revealed that none of the treatment effects were modified by missing data patterns, even though subjects that dropped out of the study due to nursing home placement or death scored markedly worse and/or deteriorated faster on all assessment scales.

Adverse effects

Caregivers provided a total of 694 adverse effects scale ratings, of which 571 follow-up assessments, from 182 subjects. Table 2 gives an overview of their occurrence. Items with the highest overall ratings were drowsiness and irritability, items with the least overall ratings stomach ache and pins and needles. Of note, none of the possible adverse effects as suggested in previous studies on light and melatonin worsened by either light or melatonin treatment or their interaction. On the contrary, as compared to the pre-treatment assessment and the placebo-treated subject, light treatment significantly lowered the ratings on irritability, dizziness, headache, obstipation and inability to sleep. Melatonin lowered the ratings on obstipation.

Discussion

This is the first double-blind, placebo-controlled randomized follow-up trial applying a combination of the circadian Zeitgebers light and melatonin on a daily basis for fifteen months on average and up to three and a half years in a limited number of cases. Such an extended period of daily light treatment is only feasible by application of indirect ceiling-mounted whole-day bright light not requiring any effort from the subjects and thus resulting in optimal compliance. Importantly, the procedure of installing new fixtures also allowed us to create both active and placebo conditions which we verified to be equally appreciated by the caregivers. As a consequence, this solved the major problem of differential expectancies between an active and placebo condition that may have affected some of the previous studies on light therapy.

The simple measure of installing high-intensity illumination in the common living rooms of mostly demented elderly residents

of group care facilities resulted in a 19% (1.5 points) reduction of depressive symptoms, even though it could not fully prevent the progressive increase of 2.1 CSDD points per year. The results on the CSDD were supported by the improvement on the PGCMS-lonely dissatisfaction subscale. The grand mean of the CSDD scores was 8.8±0.6, indicating that, on average, subjects fell in the range of episodic minor depressive disorder, which is defined as a score between 8 and 12.[29] Epidemiological studies have shown that in older subjects the presence of clinically significant depressive symptomatology is more prevalent than the diagnosis major depressive disorder.[42,43] Also in Alzheimer patients, depressive symptoms frequently occur in the absence of major depression.[4] In the present study we applied the treatment to all institutionalized demented subjects, without selection based on the presence of mood disorders. We thus studied a heterogeneous group, but with a high risk of suffering from depressive symptoms. For a considerable part, the worsening of mood over time was prevented by light treatment. Light moreover strongly attenuated the increase in limitations of activities of daily living. Thus, the effect of licht on functional limitations increased with the duration of treatment.

Melatonin had no effect on the CSDD depression scores, but adversely affected: (1) observed withdrawn behaviour (MOSES), (2) the behavioural expression of a negative mood (PGCARS-negative) and (3) the behavioural expression of a positive mood (PGCARS-positive). This adverse effect on the behavioural expression of a negative mood was counteracted when melatonin was given in combination with bright light.

One explanation for the adverse effect of melatonin on observed mood might be that the long-term daily application of 2.5 mg daily could result in sustained supra-physiological levels of circulating melatonin, even during daytime. Elevated daytime melatonin levels have been proposed to induce adverse effects, including sleepiness.[44] Indeed, increased dysphoria with high dosage melatonin has been described in non-demented depressed adults.[45] The partial reversion of the adverse effects on mood of long-term melatonin application by simultaneous bright light treatment is in line with previous observations that bright light may have alerting effects even under conditions of elevated plasma melatonin levels.[46-48] A practical implication of the observed adverse effects of long-term melatonin use on mood is that, when melatonin is administered to elderly residents of group care facilities patients to improve sleep disturbances, a dose lower than 2.5 mg should be considered as well as simultaneous application of bright light.

However, melatonin did by no means only induce adverse effects. A time by treatment by dropout pattern interaction effect on the NPI-Q, indicated that melatonin strongly attenuated the worsening of psychopathological symptoms. This effect was limited to subjects with pending institutionalization or death, in whom we found a worsening of the severity of psychopathological symptoms and caregiver distress over time, which was reduced by 62%, respectively 84% when treated with melatonin. Moreover, as indicated by the CMAI, the combined application of melatonin and bright light attenuated agitated behaviour by 9%.

One factor that has been found to interfere with therapeutic outcome in dementia is the Apolipoprotein E (ApoE) genotype.[49] As we had assessed the ApoE genotype from saliva samples of the subjects in our study, we have checked for the interaction in the dataset. We did not find an interaction between treatment outcomes on both mood and behavioural disturbances and the ApoE genotype.

According to a recent Cochrane review by Forbes et al.,[24] the quality of most studies on light and melatonin so far did not permit conclusions that could be useful to evaluate their value for the treatment of sleep, mood and behaviour disturbances in dementia in clinical practice. With the present first long-term randomized placebo-controlled follow-up study we aimed to bridge the gap between promising results from previous sub-opti-

mally controlled or small studies on the one hand and evidence-based clinical practice on the other hand. Two limitations of the present study should be discussed. First, the study was performed in a somewhat heterogeneous group of mostly demented elderly residents of group care facilities. Although the majority of the participants were diagnosed with probable Alzheimer, the study thus did not focus on a strictly defined single nosological entity. However, the sample is representative for elderly residents of group care facilities, and results can at least be generalized at least to this strongly growing population, if not also to Alzheimer's disease, since the effects reported were not modified by this diagnosis. Second, one could argue that the increasing number of subjects lost to follow-up assessment was another limitation of the present study. The drop-out was primarily due to logistic limitations, i.e. discontinuation of facilities, and secondarily related to the very nature of the population under study, which is at high risk of death and transfer to a nursing home. We verified that the treatment effects were not modulated by dropout pattern and moreover turned out to be robust in a sensitivity analysis limiting the dataset to the first 1.5 years of follow-up only. Thus, the results cannot easily be attributed to a confounding effect of the large number of dropouts.

In conclusion, our findings indicate that the simple measure of increasing the illumination level in group care facilities for mostly demented elderly considerably prevents the worsening of mood over time. In combination with daily melatonin, agitated behaviour ameliorates as well. Patients showing a worsening of neuropsychiatric symptoms can profit from melatonin treatment. However, it remains to be evaluated whether a dose smaller than 2.5 mg would still remain efficacious. At present, chronic use of melatonin by elderly can only be recommended in combination with light in order to suppress possible adverse effects on mood. The chronic application of whole-day bright light was without adverse effects and should be considered as a standard procedure for care facilities for mostly demented elderly.

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References

1. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. Lancet 2005;366(9503):2112-7.

2. Courtney C, Farrell D, Gray R, et al. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. Lancet 2004;363(9427):2105-15.

3. Lee HB, Lyketsos CG. Depression in Alzheimer's disease: heterogeneity and related issues. Biological Psychiatry 2003;54(3):353-62.

4. Purandare N, Burns A, Craig S, Faragher B, Scott K. Depressive symptoms in patients with Alzheimer's disease. International Journal of Geriatric Psychiatry 2001;16(10):960-4.

5. Gonzalez-Salvador T, Lyketsos CG, Baker A, et al. Quality of life in dementia patients in long-term care. Int J Geriatr Psychiatry 2000;15(2):181-9.

6. Donaldson C, Tarrier N, Burns A. Determinants of carer stress in Alzheimer's disease. International Journal of Geriatric Psychiatry 1998;13(4):248-56.

7. Wilson RS, Bienias JL, Mendes de Leon CF, Evans DA, Bennett DA. Negative affect and mortality in older persons. American Journal of Epidemiology 2003;158(9):827-35.

8. Dorenlot P, Harboun M, Bige V, Henrard JC, Ankri J. Major depression as a risk factor for early institutionalization of dementia patients living in the community. International Journal of Geriatric Psychiatry 2005;20(5):471-8.

9. Devanand DP, Jacobs DM, Tang MX, et al. The course of psychopathologic features in mild to moderate Alzheimer disease. Archives of General Psychiatry 1997;54(3):257-63.

10. Banerjee S, Murray J, Foley B, Atkins L, Schneider J, Mann A. Predictors of institutionalisation in people with dementia. Journal of Neurology, Neurosurgery & Psychiatry 2003;74(9):1315-6.

11. Sink KM, Holden KF, Yaffe K. Pharmacological treatment of neuropsychiatric symptoms of dementia: a review of the evidence. Jama 2005;293(5):596-608.

12. Salzman C, Wong E, Wright BC. Drug and ECT treatment of depression in the elderly, 1996-2001: a literature review. Biological Psychiatry 2002;52(3):265-84.

13. Souêtre E, Salvati E, Belugou JL, et al. Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality. Psychiatry Research 1989;28(3):263-78.

14. Van den Hoofdakker RH. Chronobiological theories of nonseasonal affective disorders and their implications for treatment. Journal of Biological Rhythms 1994;9(2):157-83.

15. Eastman CI, Young MA, Fogg LF, Liu L, Meaden PM. Bright light treatment of winter depression: a placebo-controlled trial. Archives of General Psychiatry 1998;55(10):883-9.

16. Swaab DF, Fliers E, Partiman TS. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Research 1985;342(1):37-44.

17. Zhou JN, Riemersma RF, Unmehopa UA, et al. Alterations in arginine vasopressin neurons in the suprachiasmatic nucleus in depression. Archives of General Psychiatry 2001;58(7):655-62.

18. Liu RY, Zhou JN, Hoogendijk WJG, et al. Decreased vasopressin gene expression in the biological clock of Alzheimer's disease patients with and without depression. Journal of Neuropathology & Experimental Neurology 2000;59(4):314-22.

19. Moore RY. Entrainment pathways and the functional organization of the circadian system. In: Buijs RM, Kalsbeek A, Romijn HJ, Pennartz CMA, Mirmiran M, eds. Progress in Brain Research. Amsterdam: Elsevier, 1996: 103-119.

20. Van Someren EJW, Riemersma RF, Swaab DF. Functional plasticity of the circadian timing system in old age: light exposure. Progress In Brain Research 2002;138:205-31.

21. Riemersma RF, Mattheij CAM, Swaab DF, Van Someren EJW. Melatonin rhythms, melatonin supplementation and sleep in old age. In: Straub RH, Mocchegiani E, eds. The Neuroendocrine Immune Network in Ageing. Amsterdam: Elsevier, 2004: 195-211.

22. Ancoli-Israel S, Martin JL, Kripke DF, Marler M, Klauber MR. Effect of light treatment on sleep and circadian rhythms in demented nursing home patients. Journal of the American Geriatrics Society 2002;50(2):282-9.

23. Haffmans PM, Sival RC, Lucius SA, Cats Q, van Gelder L. Bright light therapy and melatonin in motor restless behaviour in dementia: a placebo-controlled study. International Journal of Geriatric Psychiatry 2001;16(1):106-10.

24. Forbes D, Morgan D, Bangma J, Peacock S, Pelletier N, Adamson J. Light Therapy for Managing Sleep, Behaviour, and Mood Disturbances in Dementia. Cochrane Database Systematic Reviews 2004;2:CD003946.

25. American Psychiatric Association. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Washington, DC: American Psychiatric Press, 1994.

26. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34(7):939-44.

27. Middleton B, Stone BM, Arendt J. Human circadian phase in 12:12 h, 200: <8 lux and 1000: <8 lux light-dark cycles, without scheduled sleep or activity. Neuroscience Letters 2002;329(1):41-4.

28. Van Someren EJW, Kessler A, Mirmiran M, Swaab DF. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biological Psychiatry 1997;41:955-63.

29. Alexopoulos GS, Abrams RC, Young RC, Shamoian CA. Cornell scale for depression in dementia. Biological Psychiatry 1988;2:271-84.

30. Harwood DG, Ownby RL, Barker WW, Duara R. The factor structure of the Cornell Scale for Depression in Dementia among probable Alzheimer's disease patients. American Journal of Geriatric Psychiatry 1998;6(3):212-20.

31. Lawton MP. The Philadelphia Geriatric Center Morale Scale: a revision. Journal of Gerontology 1975;30(1):85-89.

32. Lawton MP, Van Haitsma K, Klapper J. Observed affect in nursing home residents with Alzheimer's disease. Journals of Gerontology B Psychological Sciences & Social Sciences 1996;51:P3-P14.

33. Helmes E, Csapo HG, Short JA. Standardization and validation of the Multidimensional Observation Scale for Elderly Subjects (MOSES). Journal of Gerontology 1987;42:395-405.

34. Kaufer DI, Cummings JL, Ketchel P, et al. Validation of the NPI-Q, a brief clinical form of the Neuropsychiatric Inventory. Journal of Neuropsychiatry & Clinical Neurosciences 2000;12(2):233-9.

35. De Jonghe JF, Kat MG, Kalisvaart CJ, Boelaarts L. [Neuropsychiatric inventory questionnaire (NPI-Q): A validity study of the Dutch form]. Tijdschrift voor Gerontologie en Geriatrie 2003;34(2):74-7.

36. Cohen-Mansfield J, Marx MS, Rosenthal AS. A description of agitation in a nursing home. Journal of Gerontology 1989;44(3):M77-M84.

37. De Jonghe JF, Kat MG. Factor structure and validity of the Dutch version of the Cohen-Mansfield Agitation Inventory (CMAI-D) [letter]. Journal of the American Geriatrics Society 1996;44(7):888-889.

38. Holmes, D. et al. Impacts associated with special care units in long-term care facilities. Gerontologist 30, 178-83 (1990).

39. Katz, S., Ford, A. B., Moskowitz, R. W., Jackson, B. A. & Jaffe, M. W. Studies of Illness in the Aged. The Index of Adl: A Standardized Measure of Biological and Psychosocial Function. Jama 185, 914-9 (1963).

40. Twisk JWR. Applied longitudinal data analysis for epidemiology. Cambridge: Cambridge University Press, 2003.

41. Siddiqui O, Ali MW. A comparison of the random-effects pattern mixture model with last-observation-carried-forward (LOCF) analysis in longitudinal clinical trials with dropouts. Journal of Biopharmaceutical Statistics 1998;8(4):545-63.

42. Blazer DG. Depression in late life: review and commentary. Journal of Gerontology 2003;58(3):249-65.

43. Beekman AT, Copeland JR, Prince MJ. Review of community prevalence of depression in later life. British Journal of Psychiatry 1999;174:307-11.

44. Zhdanova IV, Wurtman RJ, Regan MM, Taylor JA, Shi JP, Leclair OU. Melatonin treatment for age-related insomnia. Journal of Clinical Endocrinology & Metabolism 2001;86(10):4727-30.

45. Carman JS, Post RM, Buswell R, Goodwin FK. Negative effects of melatonin on depression. American Journal of Psychiatry 1976;133(10):1181-1186.

46. Deacon S, Arendt J. Adapting to phase shifts, II. Effects of melatonin and conflicting light treatment. Physiology & Behavior 1996;59(4-5):675-82.

47. Lowden A, Akerstedt T, Wibom R. Suppression of sleepiness and melatonin by bright light exposure during breaks in night work. Journal of Sleep Research 2004;13(1):37-43.

48. Ruger M, Gordijn MC, Beersma DG, de Vries B, Daan S. Weak relationships between suppression of melatonin and suppression of sleepiness/fatigue in response to light exposure. Journal of Sleep Research 2005;14(3):221-7.

49. Sjogren M, Hesse C, Basun H, et al. Tacrine and rate of progression in Alzheimer's disease--relation to ApoE allele genotype. Journal of Neural Transmission 2001;108(4):451-8.

Chapter 9

General discussion

1. *Main research question*
2. *Previous studies and specific research questions*
3. *Findings*
   - 3.1 Effects on rest-activity rhythms
   - 3.2 Effects on sleep
   - 3.3 Effects on cognition
   - 3.4 Effects on non-cognitive symptoms
     - Mood
     - Behaviour
4. *Clinical relevance and future studies*

1. The main question of this thesis was whether disturbances in sleep, mood, behaviour and cognition in, mostly demented, elderly residents of group care facilities, which are at least partly related to disturbances in the circadian timing system (CTS), could be prevented or ameliorated in a clinical relevant and applicable way by long-term reactivation of the CTS by its natural input. This hypothesis is based upon the more general hypothesis that neurons that are deprived from stimuli are at higher risk of degeneration and loss of function. In addition, when the appropriate stimulating input is given, this process can be attenuated or even reversed, which would lead to an increase in functionality of neurons.1 Central in our study is the neuronal activity of the suprachiasmatic nucleus (SCN). This is the central regulator of circadian rhythms in the human body. For optimal functionality and synchronization with the 24-hour light/dark cycle the SCN needs daily information from the environment. Light is the most important Zeitgeber transferring this information to the SCN via a pathway directly from the retina to the SCN.[2] On the other hand, there are also Zeitgebers that are primarily under circadian control of the SCN, but by their feedback they contribute to a proper function of the SCN. Examples of such Zeitgebers are the 24-hour melatonin rhythm and physical activity. In addition, these Zeitgebers can be enhanced; exogenous melatonin can be taken in tablets to supplement the evening and night endogenous excretion from the pineal gland, and physical exercise can be performed in a daytime schedule. We designed a randomized double-blind follow-up study with the main external Zeitgeber, light, and the supplementation of one of the main internal Zeitgebers, melatonin, in order to test our hypothesis in, mostly demented, elderly residents of group care facilities.

2. Previous studies and remaining research questions

Previous studies showed that light therapy is effective for the treatment of sleep-wake rhythm disturbances in demented elderly [3-8], agitated behaviour[9], depression [10] and cognition[11]. Randomized placebo-controlled trials on the effect of exogenous melatonin have demonstrated its positive effect on sleep disturbances in elderly insomniacs [12-16] and demented elderly [17]. Unanswered questions are, however, whether long-term treatment is as effective. The study duration in the above mentioned studies was 10 days till 4 weeks for light treatment[3-7, 18, 19] and 10 days till 2 months for melatonin treatment[12-17]. Since disturbances are treated that are related to the aging process, long-term application of the treatment (i.e. ranging from months till years), will be necessary for continuous stimulation of the aging biological clock. In the clinical trial described in this thesis we therefore implemented the following approaches. 1) Whole-day indirect bright light therapy was given. This way of applying light therapy takes no effort of either patients or caregivers, which is essential for the feasibility of long-term treatment application. 2) Long-term application of the treatment in a randomized controlled study, with an average follow-up of 1.3 years, with, in some cases, a maximum of 3.5 years, in order to answer the question whether light and/or melatonin will keep their effect in the long term or that the effect will be mediated by time. 3) Multiple parameters were studied allowing the investigation of the relation between sleep-wake rhythms, mood, behaviour and cognition and for evaluation of effect mediation. 4)

A large sample of subjects was studied allowing the evaluation of predictors of sensitivity to treatment. 5) Studying a group of mostly demented elderly being at risk of age-related disturbances in sleep, mood and behaviour, instead of only subjects with confirmed presence of these disturbances, allowed us to answer the question whether light and/or melatonin treatment are also able to prevent disturbances in sleep, mood and behaviour.

3. *Findings*

3.1 Effects on rest-activity rhythms

The biological clock regulates, besides other circadian rhythms, also the circadian rhythmicity of the rest-activity pattern. Rest-activity rhythm assessments, which are relatively feasible in residents elderly, can therefore contribute to the evaluation of SCN function. We found that the relative amplitude (RA) - the normalized difference of the most active 10-hour period and the least active 5-hour period - increased by 5% per year in the group receiving both light and melatonin treatment. This increase mostly resulted from a decrease in activity in the least active hours by 9% per year. There was no change in the amount of daytime activity (Chapter 7). This finding indicates that enhanced SCN input ameliorates nocturnal restlessness. The effect is not immediate but gradually develops and continues to increase over time: the longer the duration of supplementation, the larger the effect.

These findings suggest, in accordance with previous studies [5, 6, 18], that the SCN in mostly demented elderly demented subjects is still susceptible to the input that is necessary for its proper function. In other words, we confirm that there is functional plasticity in the CTS (Chapter 2). A novel finding is that the effect of light and melatonin gradually develops and continues to increase over a long follow-up period. The benefit of treatment thus increases with the duration of its application, arguing for integration of the treatment in the habitual daily life of demented elderly. The only previous study that applied whole-day bright light - in more severely disturbed demented elderly - found improvement in the rest-activity after four weeks of treatment, most of which was lost four weeks after discontinuation of the treatment [6]. Mishima et al. [5] also evaluated discontinuation effects, and found preservation of morning-light-induced effects up till two weeks after termination of treatment – a follow-up interval only half as long as in the study of Van Someren et al. [6]. These findings emphasize that it may take some time before light-induced changes become evident on the one hand, and before these changes will disappear after discontinuation of treatment on the other. Thus, it may require not only chronic exposure changes but also take time to affect the circadian timing system in demented elderly. Consequently, it appears of importance to apply treatment continuously, the feasibility of which requires integration in the habitual daily environment and routine.

3.2 Effects on actigraphic sleep estimates

The combination of light and melatonin treatment increased (SE) by 3.5 %, as compared to an efficiency of 73% for assessments made under only one or no active treatments, which is suboptimal. The combination also slowly decreased the mean duration of intermittend awakenings – likely related to their sleep-disturbing effect –by 0.5 minutes/year (12%) per year. Sleep latency, the time it takes to fall asleep after the lights are out, decreased with melatonin, by 8 min (19%). Melatonin also increased the mean duration of uninterrupted sleep periods – most likely related to sleep depth - by 6 minutes (25%), and sleep duration by 27 minutes (6%), irrespective of treatment duration. On the other hand, the additional effect of light on sleep duration developed only gradually, by 10 minutes per year. It seems that sleep disturbances are best treated by a combination of light and melatonin treatment instead of one of the treatment options. In this way there is the 'immediate' effect of melatonin - in our study within 6 weeks of treatment - and the added effect of bright light slowly developing

over time. The combination of Zeitgebers has so far not been studied in a large controlled follow-up study on sleep disturbances in dementia. Haffmans et al. studied the combination of the two therapies with regard to motor restless behaviour and suggested that the addition of melatonin to light treatment led to worse results than treatment with light only [9]. It should be noted, however, that only six patients completed the cross-over trial.

The results we found are based upon data assessed by actigraphic assessment of the sleep-wake rhythm. It is most improbable that similar results could have been found with a sleep questionnaire. In Chapter 5 we describe the comparison between an informant observed sleep questionnaire with actigraphy. One remarkable result is that none of the questionnaires of 78 subjects had all items completed. The items "snoring" and "combative or violent behaviour" were not answered affirmatively in any of the subjects. These kinds of questions can probably only be answered by a partner or caregiver sharing the same bedroom. Furthermore, the difference of caregiver estimated sleep-time and actigraphy estimated actual sleep-time was 96 minutes. Caregivers thus overestimate the actual sleep-time by more than one and a half hours on average.

3.3 Effects on cognition

In the majority of demented elderly, cognitive decline is progressive. So far, the available drugs have only been successful in slowing down disease progression, and no treatment has been found that is able to stop the disease process [20, 21].

Because cognition is an important indicator of disease progression and severity of dementia it was an important outcome measure in our study. Furthermore, sleep disturbances might add an extra burden on cognitive functioning in already cognitive impaired demented elderly [22-24]. Indeed, Moe et al. found sleep quality and cognitive performance to be related in demented elderly.[25] We therefore hypothesized that a treatment that would improve the sleep-wake rhythm disturbances might also ameliorate cognitive dysfunction, at least for the part tentatively aggravated by sleep-wake rhythm disturbances.

Although there was thus good reason to study the effects on cognitive function, the effect we found was striking even to ourselves. Light treatment had a positive, though different, effect on cognitive functioning, as indicated by a 5% higher score on the MMSE during light treatment as compared to the baseline and the dim light condition. The effect of light occurred 'instantaneously': it was already present at the first post-assessment after 6 weeks of light treatment and thereafter neither increased nor decreased in strength over time. Figure 3a in Chapter 7 moreover suggests an additional effect of melatonin, which however needed time to develop. Analysis of the complete dataset indeed suggested melatonin treatment to attenuate the annual rate of decline in MMSE by 0.6 points, which is a change of 28% in the slope of cognitive deterioration. This effect could however not be confirmed in analysis on the restricted dataset including only the first 1.5 years of follow-up, and would thus require further support from large-sample longitudinal studies with less dropout. The magnitude of the effect of light on the MMSE is comparable with the effect that can be accomplished in a younger, less affected and more homogeneous group of Alzheimer patients by means of acetylcholinesterase inhibitors, e.g. donepezil[26]. However, whereas patients treated with donepezil frequently suffer from side-effects like nausea, vomiting, diarrhea and anorexia,[27] treatment with light was without their commonly suspected adverse effects. In fact, many of the possible adverse effects occurred less in the active treatment conditions (Chapter 7). It would thus seem worthwile to investigate the relative effects of acetylcholinesterase inhibitors and chronic light treatment in a younger, less affected and more homegeneous group of Alzheimer patients.

In agreement with experimental studies [24] that have shown a contribution of sleep to daytime cognitive performance, we found a considerable co-variation of

MMSE with sleep in demented elderly, the most pronounced so with the parameter that quantifies the circadian component in the 24-hour sleep-wake cycle (Chapter 7).

We tried to measure cognitive function with the mini-mental state examination (MMSE) [28] and the cognitive part of the Alzheimer's disease assessment scale (ADAS-cog)[29]. Both scales are widely used in research studies. In our study, we could finally only use the data of the MMSE for further statistical analysis. Only a small proportion of the participants was able to complete the ADAS-cog. Due to the length of the questionnaire, only few participants were able to concentrate long enough to complete it. Because the ADAS is widely used in dementia studies, we asked ourselves what difference could account for the large proportion of missing values. One explanation could be that the ADAS was one of a battery of questionnaires in our study and the combination could therefore be too tiresome for this group of subjects. It may also be that our patients turned out to be, on average, too demented for successful assessment using the ADAS-cog.

3.4 Effects on non-cognitive symptoms

Not only cognitive deterioration but possibly even more depression and behavioural disturbances are other important and burdening symptoms that are more prevalent in demented elderly than in age-matched controls30, 31. Whereas previous work suggested a relationship with SCN dysfunction, no direct evidence of changes in SCN neurons of depressed subjects had been described in the literature. We, therefore, studied postmortem SCN tissue of 11 depressed subjects, and compared vasopressin mRNA (AVP-mRNA) expression and vasopressin immunoreactivity (AVP-IR) with 11 age- and sex-matched controls (Chapter 4). The results were surprising, and stressed the importance of combining protein expression and mRNA expression in postmortem evaluation of neuronal function. We found a more than one and a half times higher number of AVP-IR neurons in depressed subjects as compared to controls. On the other hand, the amount of AVP-mRNA was about one half of that of controls. Only in combination, these findings revealed a change in the balance between the production and transport of AVP in SCN neurons in depression. This disbalance could underlie the previously suggested functional disturbances of the SCN thought to be responsible for the circadian rhythm disturbances that have been found in depression 32, 33.

We had initially expected a decrease in the number of AVP-IR neurons. Apart from its function in circadian rhythmicity, AVP from the SCN inhibits also corticotrophin releasing hormone (CRH)-producing neurons in the paraventricular nucleus (PVN). A reduction in AVP in the SCN would contribute to the previously reported increased expression of CRH and CRH-mRNA in PVN neurons in depression34, 35. After first finding - contrary to our hypothesis - an increased number of AVP-IR neurons we, therefore, also studied AVP-mRNA as a measure of AVP production. This demonstrated a decrease in AVP-mRNA, in stark contrast with the increase in cellular peptide content. Combining these results allowed for the conclusion that AVP probably accumulates in SCN neurons. We suggest that this might be due to a decrease of the transport rate that is even stronger than the diminution in AVP production, and expect that further studies into cellular AVP transport could yield important insights in the cellular pathology associated with depression.

There is an important difference between our findings in depressed non-demented subjects, and the findings in AD patients by others groups,36-38 which suggested that both AVP-IR and the amount of AVP-mRNA are decreased. However, Liu et al. could not demonstrate a decrease in AVP-mRNA expression in AD patients, either with or without depression.36

In Chapter 6 we describe a more indirect functional connection between the circadian timing system and depression in, mostly demented, elderly residents of group care facilities. By studying the correlation between

different parameters of the rest-activity rhythm and ratings of the symptoms of depression and social interaction we found (1) that the more depressed subjects had a lower interdaily stability of the 24-hour rest-activity rhythm and (2) that the socially less interactive subjects showed less daytime activity. These results were obtained from the baseline data of our clinical trial and strengthened the belief that treatment strategies that enhance circadian rhythmicity might be beneficial to depression. Chapter 8 describes that this indeed turned out to be the case. Depressive symptoms measured with the Cornell scale for depression in demented elderly (CSDD), increased progressively by 2.1 CSSD rating points per year and an additional 1.5 points per year in the group that dropped out due to nursing home placement or death. Irrespective of time in treatment, bright light strongly improved depressive symptoms, although it could not completely prevent their gradual increase over time. Effectively, the depressive symptom ratings in the group treated with bright light stayed significantly below those of the group not receiving bright light. Melatonin had no effect on the CSDD ratings. However, it had a negative effect on positive mood items and increased negative mood items on the Philadelphia Geriatric Center Affect Rating Scale (PGCARS). The increase in negative mood was completely counteracted when melatonin was given in combination with bright light. An explanation for the adverse effect of melatonin could be that a dosage of 2.5 mg melatonin results in supraphysiological levels of melatonin which might not be cleared the following day, resulting in high daytime melatonin levels. High melatonin dosages have been proposed to induce sleepiness[39] and dysphoria[40].

Further support of the negative effect of melatonin on mood is its effect on the MOSES-withdrawn behaviour subscale. Overall, withdrawn behaviour slowly increased by more than 1 point per year. Irrespective of time in treatment, subjects receiving melatonin were rated as 1 point more withdrawn than subjects receiving the placebo tablet. Our clinical trial indicates that light is the preferable treatment of depressive symptoms in demented elderly, and that if melatonin is prescribed, it might best be tried in a dose much lower than 2.5 mg and/or in combination with bright light.

Neuropsychiatric symptoms increased by 2.1 points per year on the NPI-Q severity subscale and by 2.5 points per year on the NPI-Q caregiver distress subscale only in the subgroup of patients who dropped out of the study due to transfer to a nursing home or death. In this subgroup of patients melatonin attenuated the increase over time on severity and caregiver distress by 1.3 points and 2.1 points respectively. In other words, an improvement of the rate of change of 62% and 84% respectively. Light treatment or the combination of light and melatonin had no effect on neuropsychiatric symptoms.

Agitated behaviour, assessed by the Cohen-Mansfield Agitation Inventory (CMAI), increased in all subjects by 1.3 points per year and an additional increase of 4.2 points in the subjects who dropped out of the study due to transfer to a nursinghome or death.

Ancoli-Israel et al.[7] showed a delay of the acrophase of agitative behaviour, but they could also not find an amelioration of agitation in demented elderly by bright light treatment only. Lyketsos et al. [3] could also not find an effect of light treatment alone on agitated behaviour. Two open label trials found a positive effect of light treatment on agitation, but being an open label trial, one might criticize the reliability of these findings. [5, 41] Furthermore, from the study of Skjerve et al., one patient was excluded after the second treatment week because of an increase of agitation.

The results of our study indicate that agitated behaviour is best treated with a combination of prolonged light and melatonin supplementation.

Finally, limitations of activities of daily living increased by 20% per year and an additional 17% per year in subjects that dropped out of the study due transfer to a nursing home or death – who moreover started

at 33% higher levels of limitations. Light treatment attenuated the increase in ADL limitations, i.e. resulted in a 53% less steep decline as compared to the baseline decrease.

4. Clinical relevance and future studies

One of the main goals of the present thesis was to investigate whether the effects of light and melatonin are not only statistically significant, but also clinically relevant. Recently both light and melatonin treatment have been reviewed with regard to their therapeutic implications for clinical practice. In a systematic Cochrane review the effect of light on sleep, mood and behaviour in dementia was reviewed.[42] Forbes et al. concluded in 2004 that the quality of most studies on light and melatonin until that moment did not permit conclusions that could be useful to evaluate their value in clinical practice. Only five studies met the inclusion criteria of which only three studies could be included in the systematic review because of inappropriate reporting of the analyses and inability to retrieve data from the investigators of the other two studies. The analysis and reporting of the randomized clinical trial described in the present thesis was not yet completed at the time this Cochrane review was published. However, our trial meets all four criteria that are recommended for future studies in the review: 1. randomized controlled parallel-group design with statistically appropriate analysis, 2. a computer generated randomization technique, 3, a sample size with sufficient power to detect an effect of clinically significant magnitude and 4. blinded or objective outcome ratings [42].

Another Cochrane review, by Jansen et al., systematically investigated the use of melatonin for cognitive impairment [43]. The use of melatonin for the treatment of insomnia was also systematically reviewed, by Olde Rikkert et al [44]. The Cochrane review of Jansen et al. concluded, based on three included studies [17, 45, 46], that there is insufficient evidence to either support or reject the use of melatonin in the treatment of cognitive impairment. The duration of the reviewed studies ranged from 2-7 weeks. A novel finding that could be revealed only by the long-term character of our clinical trial, however, demonstrated a significant interaction of melatonin treatment with time. Such slow development of efficacy may have been the reason why the short-term application reported in earlier studies could not demonstrate efficacy. In their review on melatonin for the treatment of insomnia, Olde Rikkert and Rigaud concluded that more research should be carried out in less selected groups of elderly insomniacs, with a special need for large clinical trials with more circadian regulators. The clinical trial described in this thesis has fulfilled this need, thereby bringing melatonin closer to a clinically relevant drug in the treatment of insomnia.

It seems that the clinical diagnosis of dementia is not relevant for the effects we have found in our clinical trial. Analyses of the data did not reveal effect-modification by different clinical diagnoses.

In addition, our findings provide proof of principle for the idea that application of the appropriate stimuli can improve brain function even in elderly residents of group care facilities, many of which suffer from various neurodegenerative diseases. Within the limitations set by their age and/or disease, even relatively ordinary measures such as the application of light, melatonin, and possibly other regimens that enhance the stability of the sleep-wake rhythm, help to ameliorate disturbances of sleep and cognition.

We state that it is worthwhile and clinically relevant to improve indoor lightening for institutionalized elderly. For many of them it is not possible to go outdoors to receive daily bright light. They will need daily supplementation in order to maintain daily synchronization of their circadian rhythms and to ameliorate depressive symptoms. When sleep disturbances need to be treated, melatonin could be an alternative for widely prescribed benzodiazepines, without severe side-effects like frequent falling and hip fractures, and further impairment of cognitive functioning [47, 48]. However adverse effects on mood may occur

with doses of 2.5 mg given daily for a long term in the very old and mostly demented elderly residents of group care facilities.

The primary question of the present thesis was whether chronic treatment with light and/or melatonin remains effective in the long term. We can finally conclude that this is the case and that many effects even increase with the duration of treatment. These findings strongly suggest that continued stimulation of the biological clock of demented elderly is needed to keep it ticking loud enough to be heard by the brain systems involved in the regulation of sleep, mood and cognition.

However, is chronic treatment with light and/or melatonin also "clinically relevant"? Although improving the methodology (i.e. long-term, double blind, randomized, large sample size) might have increased the reliability of the effect estimates, this does not automatically implicate that the results are clinically relevant. In other words, statistically significant results are not necessarily clinically relevant results, in terms of magnitude of the effect. On the other hand, also the magnitude of the effect of acetylcholinesterase inhibitors in dementia is not very large, though the effects are significant and they are approved for the treatment of Alzheimer's disease [20]. In the case of dementia, progression of the disease takes years, which implicates that a positive treatment effect ideally should last up to the end of disease progression. With respect to this point, we judge the finding that some of the effects show a significant interaction of the treatment with time, as clinically relevant. Another important point is that we choose a heterogeneous group of demented subjects so that the application of the results is not limited to a small subgroup of the demented population. This fulfils also the need for a practical clinical trial, as stated by Tunis et al.[49] Whether treatment with light or melatonin is cost-effective still needs to be studied. It certainly will add to the clinical relevance of the treatment if this would be the case.

Future research should address the question whether a lower dosage of melatonin will be as effective in this group of subjects, without the negative effect on mood. Some studies already showed that a lower dosage of 0.3-1 mg is as effective as a higher dose of up to 6 mg [39, 50, 51]. Whether a low dose of melatonin treatment at the long-term is as effective on sleep-wake rhythm disturbances in demented elderly, without the side effects on mood, needs to be studied. Moreover - the slowly developing effect of melatonin on cognition, present in the 3.5 year analysis, but not confirmed if restricting the analysis to the first 1.5 years – deserves attention in future long-term follow-up trials.

Another important step in future research would be to study a group of mildly demented patients or subjects with mild cognitive impairment. In this way, it might be possible to answer the question whether the sleep-wake rhythm disturbances and cognitive disturbances that were already present in the group studied in our clinical trial, could be prevented. At least it would be worthwhile to see whether it is possible to slow down the disease process at an earlier stage.

References

1. Swaab, D. F. et al. Brain aging and Alzheimer's disease; use it or lose it. Prog Brain Res 138, 343-73 (2002).
2. Moore, R. Y. Entrainment pathways and the functional organization of the circadian system. Prog Brain Res 111, 103-19 (1996).
3. Lyketsos, C. G., Lindell Veiel, L., Baker, A. & Steele, C. A randomized, controlled trial of bright light therapy for agitated behaviors in dementia patients residing in long-term care. Int J Geriatr Psychiatry 14, 520-5 (1999).
4. Mishima, K., Hishikawa, Y. & Okawa, M. Randomized, dim light controlled, crossover test of morning bright light therapy for rest-activity rhythm disorders in patients with vascular dementia and dementia of Alzheimer's type. Chronobiol Int 15, 647-54 (1998).
5. Mishima, K. et al. Morning bright light therapy for sleep and behavior disorders in elderly patients with dementia. Acta Psychiatr Scand 89, 1-7 (1994).
6. Van Someren, E. J., Kessler, A., Mirmiran, M. & Swaab, D. F. Indirect bright light improves circadian rest-activity rhythm disturbances in demented patients. Biol Psychiatry 41, 955-63 (1997).
7. Ancoli-Israel, S. et al. Effect of light on agitation in institutionalized patients with severe Alzheimer disease. Am J Geriatr Psychiatry 11, 194-203 (2003).
8. Fetveit, A., Skjerve, A. & Bjorvatn, B. Bright light treatment improves sleep in institutionalised elderly--an open trial. Int J Geriatr Psychiatry 18, 520-6 (2003).
9. Haffmans, P. M., Sival, R. C., Lucius, S. A., Cats, Q. & van Gelder, L. Bright light therapy and melatonin in motor restless behaviour in dementia: a placebo-controlled study. Int J Geriatr Psychiatry 16, 106-10 (2001).
10. Sumaya, I. C., Rienzi, B. M., Deegan, J. F., 2nd & Moss, D. E. Bright light treatment decreases depression in institutionalized older adults: a placebo-controlled crossover study. J Gerontol A Biol Sci Med Sci 56, M356-60. (2001).
11. Graf, A. et al. The effects of light therapy on mini-mental state examination scores in demented patients. Biol Psychiatry 50, 725-7 (2001).
12. Garfinkel, D., Laudon, M., Nof, D. & Zisapel, N. Improvement of sleep quality in elderly people by controlled-release melatonin. Lancet 346, 541-4 (1995).
13. Garfinkel, D., Laudon, M. & Zisapel, N. Improvement of sleep quality by controlled-release melatonin in benzodiazepine-treated elderly insomniacs. Archives of Gerontology & Geriatrics 24, 223-231 (1997).
14. Haimov, I. et al. Melatonin replacement therapy of elderly insomniacs. Sleep 18, 598-603. (1995).
15. Hughes, R. J., Sack, R. L. & Lewy, A. J. The role of melatonin and circadian phase in age-related sleep-maintenance insomnia: assessment in a clinical trial of melatonin replacement. Sleep 21, 52-68 (1998).
16. Jean-Louis, G., Von Gizycki, H. & Zizi, F. Melatonin effects on sleep, mood, and cognition in elderly with mild cognitive impairment. J Pineal Res 25, 177-183 (1998).
17. Singer, C. et al. A multicenter, placebo-controlled trial of melatonin for sleep disturbance in Alzheimer's disease. Sleep 26, 893-901 (2003).
18. Ancoli-Israel, S., Martin, J. L., Kripke, D. F., Marler, M. & Klauber, M. R. Effect of light treatment on sleep and circadian rhythms in demented nursing home patients. J Am Geriatr Soc 50, 282-9 (2002).
19. Yamadera, H. et al. Effects of bright light on cognitive and sleep-wake (circadian) rhythm disturbances in Alzheimer-type dementia. Psychiatry Clin Neurosci 54, 352-3 (2000).
20. Birks, J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev, CD005593 (2006).
21. Caselli, R. J., Beach, T. G., Yaari, R. & Reiman, E. M. Alzheimer's disease a century later. J Clin Psychiatry 67, 1784-800 (2006).
22. Drosopoulos, S., Wagner, U. & Born, J. Sleep enhances explicit recollection in recognition memory. Learn Mem 12, 44-51 (2005).

23. Wagner, U., Gais, S., Haider, H., Verleger, R. & Born, J. Sleep inspires insight. Nature 427, 352-5 (2004).

24. Walker, M. P. & Stickgold, R. Sleep-dependent learning and memory consolidation. Neuron 44, 121-33 (2004).

25. Moe, K. E., Vitiello, M. V., Larsen, L. H. & Prinz, P. N. Symposium: Cognitive processes and sleep disturbances: Sleep/wake patterns in Alzheimer's disease: relationships with cognition and function. J Sleep Res 4, 15-20 (1995).

26. Courtney, C. et al. Long-term donepezil treatment in 565 patients with Alzheimer's disease (AD2000): randomised double-blind trial. Lancet 363, 2105-15 (2004).

27. Birks, J. S. & Harvey, R. Donepezil for dementia due to Alzheimer's disease. Cochrane Database Syst Rev, CD001190 (2003).

28. Folstein, M. F., Folstein, S. E. & McHugh, P. R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12, 189-98 (1975).

29. Mohs, R. C. & Cohen, L. Alzheimer's Disease Assessment Scale (ADAS). Psychopharmacol Bull 24, 627-8 (1988).

30. Gonzalez-Salvador, T. et al. Quality of life in dementia patients in long-term care. Int J Geriatr Psychiatry 15, 181-9 (2000).

31. Purandare, N., Burns, A., Craig, S., Faragher, B. & Scott, K. Depressive symptoms in patients with Alzheimer's disease. Int J Geriatr Psychiatry 16, 960-4 (2001).

32. Souetre, E. et al. Circadian rhythms in depression and recovery: evidence for blunted amplitude as the main chronobiological abnormality. Psychiatry Res 28, 263-78 (1989).

33. Van den Hoofdakker, R. H. Chronobiological theories of nonseasonal affective disorders and their implications for treatment. J Biol Rhythms 9, 157-83 (1994).

34. Raadsheer, F. C., Hoogendijk, W. J., Stam, F. C., Tilders, F. J. & Swaab, D. F. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology 60, 436-44 (1994).

35. Raadsheer, F. C. et al. Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. Am J Psychiatry 152, 1372-6 (1995).

36. Liu, R. Y. et al. Decreased vasopressin gene expression in the biological clock of Alzheimer disease patients with and without depression. J Neuropathol Exp Neurol 59, 314-22 (2000).

37. Stopa, E. G. et al. Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. J Neuropathol Exp Neurol 58, 29-39 (1999).

38. Swaab, D. F., Fliers, E. & Partiman, T. S. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. Brain Res 342, 37-44 (1985).

39. Zhdanova, I. V. et al. Melatonin treatment for age-related insomnia. J Clin Endocrinol Metab 86, 4727-30 (2001).

40. Carman, J. S., Post, R. M., Buswell, R. & Goodwin, F. K. Negative effects of melatonin on depression. Am J Psychiatry 133, 1181-6 (1976).

41. Skjerve, A. et al. Improvement in behavioral symptoms and advance of activity acrophase after short-term bright light treatment in severe dementia. Psychiatry Clin Neurosci 58, 343-7 (2004).

42. Forbes, D. et al. Light therapy for managing sleep, behaviour, and mood disturbances in dementia. Cochrane Database Syst Rev, CD003946 (2004).

43. Jansen, S. L., Forbes, D. A., Duncan, V. & Morgan, D. G. Melatonin for cognitive impairment. Cochrane Database Syst Rev, CD003802 (2006).

44. Olde Rikkert, M. G. & Rigaud, A. S. Melatonin in elderly patients with insomnia. A systematic review. Z Gerontol Geriatr 34, 491-7 (2001).

45. Asayama, K. et al. Double blind study of melatonin effects on the sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. J Nippon Med Sch 70, 334-41 (2003).

46. Serfaty, M., Kennell-Webb, S., Warner, J., Blizard, R. & Raven, P. Double blind randomised placebo controlled trial of low dose melatonin for sleep disorders in dementia. Int J Geriatr Psychiatry 17, 1120-7 (2002).

47. Petrovic, M., Mariman, A., Warie, H., Afschrift, M. & Pevernagie, D. Is there a rationale for prescription of benzodiazepines in the elderly? Review of the literature. Acta Clin Belg 58, 27-36 (2003).

48. Wagner, A. K. et al. Benzodiazepine use and hip fractures in the elderly: who is at greatest risk? Arch Intern Med 164, 1567-72 (2004).

49. Tunis, S. R., Stryer, D. B. & Clancy, C. M. Practical clinical trials: increasing the value of clinical research for decision making in clinical and health policy. Jama 290, 1624-32 (2003).

50. Laakso, M. L., Lindblom, N., Leinonen, L. & Kaski, M. Endogenous melatonin predicts efficacy of exogenous melatonin in consolidation of fragmented wrist-activity rhythm of adult patients with developmental brain disorders: A double-blind, placebo-controlled, crossover study. Sleep Med 8, 222-39 (2007).

51. Zhdanova, I. V., Wurtman, R. J., Morabito, C., Piotrovska, V. R. & Lynch, H. J. Effects of low oral doses of melatonin, given 2-4 hours before habitual bedtime, on sleep in normal young humans. Sleep 19, 423-31 (1996).

Summary

The research described in the present thesis focuses on frequent problems in dementia which all put a high burden on the quality of life of both, the demented patients themselves and their caregivers. These problems not only consist of cognitive disabilities, but also of disturbances in behaviour, mood and the circadian regulation of sleep, hormones and temperature.

The general hypothesis studied in this thesis is that deterioration of the suprachiasmatic nucleus (SCN) is for a large part responsible for disturbed functionality of the circadian timing system (CTS), and contributes to disturbances in sleep, mood, behaviour and cognition in demented elderly. Moreover, we hypothesize that reactivation of the biological clock by the proper stimuli will improve the functionality of the biological clock, or prevent its deterioration. According to this hypothesis, enhanced exposure to the stimuli to which the biological clock is most sensitive, i.e. bright light and melatonin, should be a rational method to ameliorate sleep disturbances and to improve mood, behavioural and cognitive disturbances, at least as far as they are under the influence of the CTS.

Chapter 2 reviews how age-related degeneration in the core of the CTS, the suprachiasmatic nucleus, and the resulting weak or altered output of the system, have negative implications for health, sleep, mood, cognitive performance and overall well-being of healthy and demented elderly. Given these negative consequences, it is of importance to investigate which mechanisms underlie the degeneration of the CTS in order to be able to develop rational treatment strategies. We demonstrate how aging can be paralleled by a diminishing input of environmental light to the CTS. This condition of chronically decreased activation could contribute to neuronal shrinkage and consequently a poor expression of rhythms, paraphrased as 'use it or lose it'. We review the studies that generally confirm that bright light contributes to the expression of rhythms in core body temperature, melatonin and sleep-wakefulness in healthy and demented elderly.

Chapter 3 reviews age-related changes in the major internal stimulus to the CTS, the pineal hormone melatonin. The circadian rhythm in melatonin production is, in a feedback system, regulated by the SCN itself, resulting in low daytime circulating levels of melatonin and an increase of circulating melatonin after darkness onset. The relation between the age-related changes in melatonin levels and sleep is discussed in this chapter.

Based on the hypothesis that there is a relationship between the increased prevalence of sleep-disturbances and decreased night-time melatonin levels in elderly, several studies have been performed to investigate the effect of exogenous melatonin supplementation on sleep in elderly and demented subjects. An overview of these findings is presented and the various results are discussed. We concluded that there is the need for larger studies before widespread use of melatonin in sleep disorders can be advocated. These studies should have the statistical power to elucidate which subgroups of patients can be expected to show a positive effect of melatonin and which cannot.

Chapter 4 focuses on the functional neurobiology of the central pacemaker in major depression, an other condition in which the CTS is disturbed. Data of a post-mortem study are presented, comparing vasopressin (AVP) immunoreactivity and AVP m-RNA in the SCN between depressed and non-depressed subjects. We found that in depressed subjects, the number of AVP-IR neurons in the SCN was more than one and one-half times higher than in controls, while the total masked area of silver grains, as an estimate of the amount of AVP-mRNA, was about one half that of controls. These findings suggest that in depressed patients the transport of AVP is even more reduced than

its synthesis in the SCN, resulting in an impaired functional ability of the CTS.

Chapter 5 presents a study investigating the relation of two types of measurement of sleep disturbances; i.e. actigraphy and a questionnaire, the Circadian Sleep Inventory for Normal and Pathological States (CSINAPS). We found good correlations between the questionnaire items about habitual timing of sleep and wakefulness and their actigraphic counterparts. However, caregivers overestimated the actual time between sleep onset and offset by more than 1.5 hours. The assessment of sleep and wake disturbances in demented elderly by the CSINAPS can be best performed by the parallel use of actigraphy. Though, if sleep-disordered breathing and leg movements are a focus of interest, additional assessments are needed.

Chapter 6 describes the association between actigraphic estimates of the sleep-wake rhythm and a range of functional domains that contribute to well-being in demented elderly patients.

Cognitive, functional, behavioural and emotional states showed moderately strong correlations with multiple rhythm variables. Partial correlations indicated that this could not only be attributed to a uniform worsening with advancing cognitive decline. Stepwise regressions indicated three most distinctive rhythm variables: (1) the interdaily stability of the 24-hour rhythm was most strongly, negatively, related to cognitive decline and depression; (2) the median level of daytime activity was most strongly, negatively, related to impairments of function, of activities of daily living, and of social interaction; (3) nocturnal restlessness was secondarily, positively, related to impairments of function and of social interaction. This raises the possibility that treatments that enhance daytime activity and the stability of the rest-activity rhythm may improve these components of well-being.

Chapter 7 presents the results of long-term (up to 3.5 years) daily supplementation of the circadian synchronisers light ($\pm$ 1000 lux) and/or melatonin (2.5 mg) on cognition and actigraphic estimates of sleep-wake rhythms of 189 mostly demented elderly residents of group facilities in 12 nursing homes. This first study on long-term stimulation of the human CTS showed that prolonged administration of combined melatonin and whole-day bright light treatment in mostly demented elderly residents of group facilities, induces effects that in combination can be regarded as clinically relevant. A further important finding is that the improvement of the sleep-wake rhythm contributed to attenuation of cognitive decline, with an effect size comparable to the effect that acetylcholinesterase inhibitors have been reported to bring about in a younger, less affected and more homogeneous group of Alzheimer patients.

Chapter 8 reports on the non-cognitive outcomes of long-term application of bright light and/or melatonin in mostly demented elderly residents of group facilities. Light ameliorated depressive symptoms by 19% and limitations of activities of daily living by 11%. Melatonin ameliorated the severity and distress of psychiatric symptoms in subjects about to drop out of the study due to nursing home placement or death by 26% per year and 45% per year respectively. However, melatonin adversely influenced the ratings for positive and negative observed affect, and for withdrawn behaviour. These adverse effects were partly counteracted if melatonin was given in combination with light. The combined treatment furthermore attenuated aggressive behaviour by 9%.

Chapter 9 discusses the findings of the studies described in the thesis and the answered and unanswered research questions. The primary question of the present thesis was whether chronic treatment with light and/or melatonin

remains effective in the long term.

We can finally conclude that this is the case and that most effects even increase with the duration of treatment. These findings strongly suggest that continued stimulation of the biological clock of demented elderly is needed to keep it ticking loud enough to be heard by the brain systems involved in the regulation of sleep, mood and cognition.

Future research should address the question whether a lower dose of melatonin would be as effective on the long-term, but without the adverse effect on mood found with a daily dose of 2.5 mg. For the time being, however, the best long-term effect can be obtained with the combination of 1000 lux light and 2.5 mg melatonin per day.

Nederlandse samenvatting

In dit proefschrift wordt onderzoek beschreven naar veelvuldig voorkomende problemen bij dementie die vaak een zware wissel leggen op de kwaliteit van leven van zowel de dementerende als de verzorgers. Het gaat hierbij niet alleen om cognitieve problemen, maar ook om stoornissen in de stemming, het gedrag en de circadiane regualtie van slaap, hormonen en temperatuur.

De centrale hypothese die werd bestudeerd is dat degeneratie van de suprachiasmatische kern (SCN) bij demente ouderen voor een groot deel verantwoordelijk is voor verstoringen in het circadiane regulatie systeem (CTS) en daarmee bijdraagt aan verstoringen in het slaap-waakritme, stemming, gedrag en cognitie. Daarbij veronderstelden wij dat reactivatie van de SCN door de benodigde stimuli het functioneren zouden kunnen verbeteren, of deze zelfs zouden kunnen voorkomen. Volgend uit deze hypothese zou toediening van de stimuli waarvoor de SCN het meest gevoelig is, een rationele behandelings methode kunnen zijn om slaapstoornissen te verminderen en stoornissen in de stemming, het gedrag en cognitite te verbeteren, tenminste voor dat deel dat wordt beïnvloed door het circadiane systeem.

Hoofdstuk 2 geeft een overzicht van hoe verouderingsgerelateerde degeneratie van de SCN en de daaruit voortvloeiende verzwakte of veranderde "output" negatieve consequenties hebben voor gezondheid, slaap, stemming, cognitief functioneren en algeheel welbevinden van gezonde en demente ouderen. Gegeven deze consequenties is het van belang de onderliggende mechanismes die ten grondslag liggen aan de genoemde degeneratie te onderzoeken om zodoende therapeutische strategieën te ontwikkelen. We laten zien dat veroudering is gepaard kan gaan met een verminderde blootstelling aan omgevingslicht. Deze toestand van een chronisch verminderde activatie zou neuronale atrofie kunnen bevorderen en zodoende kunnen leiden tot een verminderde expressie van circadiane ritmes. We bespreken studies die in het algemeen bevestigen dat helder licht bijdraagt aan de expressie van circadiane ritmes in lichaamstemperatuur, melatonine en slaap-waak regulatie in gezonde en demente ouderen.

Hoofdstuk 3 geeft een overzicht van verouderings-gerelateerde veranderingen in de belangrijkste interne stimulus voor het CTS, het door de pijnappelklier geproduceerde hormoon melatonine. Het circadiane ritme in de melatonine productie staat in een teruggekoppeld systeem zelf ook weer onder controle van de SCN, hetgeen resulteert in lage waardes overdag en een stijging in de avond en nacht. De relatie tussen verouderingsgerelateerde veranderingen in melatonine spiegels en slaap wordt hier bediscussieerd.

Gebaseerd op de hypothese dat er een relatie bestaat tussen een toename van slaapstoornissen en afgenomen nachtelijke melatonine spiegels bij ouderen, hebben diverse studies gekeken naar het effect van exogeen melatonine op slaap bij ouderen en demented ouderen. Dit hoofdstuk geeft een overzicht van de verschillende resultaten van deze studies. Wij concluderen dat er een noodzaak bestaat tot het uitvoeren van grotere studies voordat melatonine aan grote groepen kan worden voorgeschreven voor de behandeling van slaapstoornissen. Deze studies zouden voldoende statistisch onderscheidingsvermogen moeten hebben om te achterhalen van welke subgroepen van patienten verwacht kan worden dat ze baat zouden kunnen hebben bij behandeling met melatonine.

Hoofdstuk 4 richt zich op de functionele neurobiologie van de SCN bij depressie, een andere conditie waarbij het CTS verstoord is. De resultaten van een post-mortem studie worden gepresenteerd, waarin vasopressine (AVP) immunoreactiviteit (IR) en AVP-mRNA in de SCN worden vergeleken tussen depressieve en niet depressieve personen. We vonden dat bij depressieve personen, het aantal AVP-IR neuronen in de SCN meern dan anderhalf keer zo groot was dan bij niet depressieve personen, terwijl de hoeveelheid AVP-mRNA ongeveer de helft kleiner

was dan bij niet depressieve personen. Deze resultaten suggereren dat bij depressie het transport van AVP meer gereduceerd is dan de productie, resulterend in een verstoorde functionaliteit van het CTS.

Hoofdstuk 5 beschrijft een studie naar de relatie tussen twee manieren om slaapstoornissen te bepalen; i.e. actigrafie en een vragenlijst, de Circadian Sleep Inventory for Normal and Pathological States (CSINAPS). Wij vonden goede correlaties tussen de items *gewoonlijke bedtijd* en *gewoonlijke ontwakingstijd* van de CSINAPS en de respectievelijke overeenkomstige waardes gemeten met actigrafie. Desalniettemin overschatten verzorgers de slaapduur tussen bedtijd en ontwakingstijd met meer dan 1,5 uur. Het bepalen van slaap-waak stoornissen bij demente ouderen met behulp van de CSINAPS vragenlijst is het meest gebaat bij het gelijktijdig gebruik van actigrafie. Echter, als slaap-apneu syndroom en onrustige benen centraal staan dan zijn additionele meetinstrumenten nodig.

Hoofdstuk 6 beschrijft de relatie tussen het slaap-waak ritme, bepaald middels actigrafie, en een serie van functionele domeinen die bijdragen aan de kwaliteit van leven van de demente ouderen. Cognitie, algemeen functioneren, gedrag en stemming lieten een redelijk sterke correlatie zien met meerdere variabelen van het slaap-waak ritme. Partiële correlaties lieten zien dat dit niet slechts toe te schrijven is aan een globale achteruitgang bij voortschrijdende cognitieve achteruitgang. Stapsgewijze regressie analyse onderscheidde 3 aparte ritme variabelen: 1. de *interdaily stability* (IS) van het 24-uurs ritme was het sterkst negatief gecorreleerd met cognitieve achteruitgang en depressie; 2. het mediane niveau van activiteit overdag was het sterkst negatief gecorreleerd met stoornissen in het algemeen functioneren, activiteiten van het dagelijks leven en sociale interactie; 3. nachtelijke onrust was secundair positief gecorreleerd met stoornissen in het algemeen functioneren en met sociale interactie. Dit suggereert de mogelijkheid dat een behandeling die activiteit overdag en de stabiliteit van het rust-acitiviteitsritme verbeteren, eveneens kunnen bijdragen aan deze componenten van de kwaliteit van leven.

Hoofdstuk 7 laat de effecten zien van lange termijn (tot 3,5 jaar) dagelijkse behandeling met de circadiane *Zeitgebers* licht (± 1000 lux) en melatonine (2,5 mg) op cognitie en met behulp van actigrafie bepaalde maten voor het slaap-waakritme bij 189 voornamelijk demente ouderen in 12 verzorgingshuizen. Deze eerste studie naar de lange termijn stimulatie van het humane CTS laat zien dat langdurige gecombineerde behandeling met licht en melatonine bij voornamelijk demente bewoners van groepszorg-faciliteiten effecten teweeg brengt die in combinatie als klinisch relevant kunnen worden aangemerkt. Een verdere belangrijke bevinding is dat de verbetering in het slaap-waak ritme bijdraagt aan een afname van de cognitieve achteruitgang, waarbij de effectgrootte vergelijkbaar lijkt te zijn met de effectgrootte die anderen vonden bij gebruik van acetylcholinesterase remmers bij jongere, lichter gedementeerde en meer homogene groepen Alzheimer patienten.

Hoofdstuk 8 beschrijft de resultaten van lange termijn behandeling met licht en melatonine op de niet-cognitieve symptomen bij voornamelijk demente bewoners van groepszorg-faciliteiten. Helder licht verminderde depressieve symptomen met 19% en beperkingen van het dagelijks functioneren met 11%. Melatonine verminderde de ernst en belasting van psychiatrische symptomen met respectievelijk 26% per jaar en 45% per jaar in de groep van mensen die zouden uitvallen door overlijden of overplaatsing naar een verpleeghuis. Melatonine had echter een negatief effect op de positieve en negatieve items van geobserveerde stemming en terugtrek gedrag. Deze nadelige effecten werden gedeeltelijk tegengegaan indien melatonine werd gecombineerd met helder licht. De gecombineerde behandeling verminderde bovendien aggressief gedrag met 9%.

Hoofdstuk 9 bediscussieert de resultaten van de studies die in het proefschrift staan beschreven en daarbij de beantwoorde en onbeantwoorde vragen. De centrale vraag in dit proefschrift was of langdurige behandeling met helder licht en/of melatonine effectief blijft op lange termijn. We kunnen concluderen dat dit inderdaad het geval is en dat voor een belangrijk deel van de effecten geldt dat de grootte van het effect zelfs toeneemt met tijd. De resultaten wijzen er op dat continue stimulatie van de biologische klok bij demente ouderen noodzakelijk is om deze sterk genoeg te laten tikken om gehoord te worden door de hersensystemen die betrokken zijn bij de regulatie van slaap, stemming en cognitie.

Toekomstig onderzoek zou moeten uitwijzen of een lagere dosering melatonine even effectief is, zonder de negatieve uitwerking op de stemming te hebben die werd gevonden bij een dagelijkse dosis van 2.5 mg. Tot deze vraag is beantwoord kan het beste lange termijn effect verwacht worden van een combinatie van 1000 lux helder licht met 2,5 mg melatonine per dag.

Dankwoord

En dan, aan het einde van een flinke periode waarin dit proefschrift tot stand is gekomen, is het tijd om stil te staan bij de mensen die met hun bijdrage dit resultaat hebben mogelijk gemaakt.

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Curriculum vitae

Rixt Riemersma werd op 4 juni 1972 geboren in Leeuwarden. Nadat zij haar middelbare school opleiding volgde aan de Vrije School Groningen, behaalde zij in 1991 haar VWO-diploma aan het Noordelijk Avond College in Groningen. Gedurende de twee jaar dat zij voor de studie geneeskunde was uitgeloot werkte zij achtereenvolgens bij de Stichting Gaudeamus, een stichting voor hedendaagse muziek en als au-pair in Brussel. In 1993 werd zij ingeloot en begon zij de studie geneeskunde aan de Universiteit van Amsterdam.

Gedurende haar studie werkte zij als administratief medewerkster en als obductie assistent bij de Nederlandse Hersenbank. Haar wetenschappelijke stage voerde zij uit aan het Nederlands Instituut voor Hersenonderzoek (het huidige Nederlands instituut voor Neurowetenschappen), de resultaten hiervan staan beschreven in hoofdstuk 4.

Na een stage van een half jaar aan het Max Planck Instituut voor Psychiatrie in München en het behalen van haar doctoraal diploma in 1998 startte zij met haar promotieonderzoek aan het Nederlands Instituut voor Hersenonderzoek. Gedurende anderhalf jaar werkte zij hier full time aan en vervolgens in afwisseling met de co-schappen. Nadat zij in 2004 haar arts-examen behaalde begon zij in 2005 met de opleiding tot psychiater aan het Universitair Medisch Centrum Groningen.

Zij is sinds 2004 getrouwd met Jelger van der Lek.