SLEEP-WAKE
Research in The Netherlands

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ABSTRACTS

PREFACE

In 2008, the NSWO Board invested much effort in the organization of the first International Sleep Medicine Course, held November 11th – 14th, 2008 in NH Conference Centre Leeuwenhorst. The course covered the essential basic and applied aspects of sleep disorders and was intended for university graduates who wanted to pursue a clinical career in sleep medicine. In total 62 participants and an international faculty of 38 speakers attended this four-day residential course. The course was subdivided into eight daily periods, each consisting of four to five lectures and one workshop, and dedicated to one of the following topics: sleep physiology, sleep monitoring, sleep disordered breathing, insomnia, circadian rhythm sleep disorders, sleep related movement disorders, hypersomnia, and parasomnias. The course was concluded with a selected set of 'clinical pearls'.

The participants rated the overall quality of the course with an average report mark of 8.1 (on a scale of 1-10), and expressed a high interest in the possibility of an advanced course. In 2009 the second edition of this ISMC will be organized by our British colleagues in Cambridge, in the last week of September.

On November 28th, 2008 the autumn meeting of the NSWO was hosted by dr. Judith Haffmans in the PsyQ building of the Parnassia Bavo Group in The Hague. The central theme of this very well attended meeting was "Pharmacology and endocrinology of sleep" and was discussed in the morning by six speakers, who presented talks about orexins, pharmacological animal studies, pharmacological treatment of sleep apnea and insomnia, and melatonin. In the afternoon, four speakers presented their studies on aging, addictive drugs, memory consolidation, and depression, respectively. During the meeting the 19th volume of Sleep-Wake Research in The Netherlands, including 23 short papers which were edited by Gé Ruigt and other members of the scientific committee, was presented. Sanofi-Aventis Netherlands BV was gratefully acknowledged for financing the publication of this volume.

The fifth National Sleep Day was held on March 28th 2009, and was organized by the PR committee around the theme "Sleep Medication". A press information file was prepared, the power point presentation of the previous year was adapted and distributed among the 34 sleep centers and research institutes of our country, and a web-based questionnaire was launched.

On March 27th 2009 the 11th edition of the annual clinical symposium 'Epilepsy and Sleep Update@Kempenhaeghe.nl' was held, including sessions with topics "Sleep disordered breathing", "New developments in basic sleep research" and "Diagnostics in sleep medicine – new frontiers", organized jointly by BASS and NSWO. The mix of excellent talks, a perfect organization and a large attendance made this a memorable day.

Gerard Kerkhof, president

EDITORIAL NOTE

This year's proceedings of the Dutch Society for Sleep Wake Research continues the Theses section that started last year. In this section, young sleep researches present the contents of their recently defended PhD theses. This year, Julia van den Berg, Birgit Koch and Tim Leufkens are the ones that describe their PhD work. I am very happy that Ton Coenen, Elsbeth Nagtegaal and Annemiek Vermeeren were willing to provide a commentary to go along with the thesis summaries.

The proceedings are intended to give a broad overview of sleep research being performed in the Netherlands. Minipapers describing original research have formed the main body of the proceedings for many years. This year, we have decided to include research abstracts as well. We hope that this will further lower the threshold for members to submit their work. After a mini-review by Tom Coenen, you will find eleven mini-papers on a wide range of subjects, followed by 26 abstracts.

The member list has been updated, and is also available on the NSWO website (www.nswo.nl). The overview of academic sites and clinics has been removed from the printed proceedings, but will be available on the website.

On behalf of the scientific committee, I would like to thank all NSWO members for their contributions. As always, many thanks to my co-editors for reviewing the manuscripts, ensuring the highest quality possible.

Sebastiaan Overeem

Chair Scientific Committee
Chief Editor NSWO Proceedings

IN MEMORIAM PROF. DR. PIET VISSER (1919-2009)

Op 23 februari 2009 overleed Prof. Dr. Piet Visser, erelid van de NSWO en een icoon van het slaaponderzoek in Nederland.

Piet was een veelzijdig en zorgzaam persoon. Hij was vooral stimulator en geen gangbare bestuurder of 'prof'. Het feit dat hij van oorsprong arts was vergat hij nooit, hoewel hij een groot deel van zijn werkzame leven hoogleraar was bij Psychofysiologie, een onderdeel van de Psychologie. Tegenover collega-psychologen verklaarde Piet dan verontschuldigend na een vlijmscherp commentaar op een psychologisch stukje: "ik ben slechts een arts".

Na zijn opleiding tot fysioloog in Leiden werd Piet Visser in 1954 benoemd tot Wetenschappelijk Ambtenaar 1e klasse aan het Lab voor Fysiologie van de Universiteit van Amsterdam. Enkele jaren later werd de arts Visser belast met het fysiologie onderwijs aan de psychologen. In 1967 werd hij benoemd tot hoogleraar in de Psychofysiologie aan de UvA, naast een hoogleraarschap in Brussel. Tot zijn emeritaat in 1984 gaf Piet Visser met veel enthousiasme leiding aan 'zijn' Laboratorium voor Psychofysiologie.

Dat de psychofysiologie alleen tot bloei zou kunnen komen met een multidisciplinaire benadering was zijn heilige overtuiging. Naast psychologen bevolkten fysici, ingenieurs, bewegingswetenschappers en biologen "zijn Lab". Hij stimuleerde zijn stafleden om te doen wat hen het meest na aan het hart lag, als het maar ten goede kwam aan psychofysiologische inzichten van ons gedrag. Hij vocht in 1970 voor de komst van de eerste computer die de Subfaculteit Psychologie rijk was, iets wat door veel psychologen met wantrouwen èn jaloezie bekeken werd. De gehele staf moest, al of niet vrijwillig, een computercursus volgen.

Zijn multidisciplinaire interesse en zorgzame persoonlijkheid hebben geleid tot een samenwerking tussen de Tsjechische fysioloog (in de tijd van het ijzeren gordijn!) Peñáz en de Nederlandse ingenieur Wesseling. Het hieruit voortgekomen unieke apparaat om continu en op niet-invasieve wijze bloeddruk te meten is ook zeer belangrijk gebleken voor cardiovasculair slaaponderzoek.

Piet Visser zei altijd van zichzelf tot het genus mensen te behoren dat in horizontale toestand slaapt en in verticale praat. Hij schroomde dan ook niet om zich als proefpersoon aan te bieden bij de proefregistraties voor het slaap-droom onderzoek dat op zijn instigatie in 1973 in zijn lab van start ging. Dat kwam de student-onderzoekers achter de kersverse registratie apparatuur vaak ook wel wat beter uit. De professor was zo enthousiast dat hij nog wel eens aan verschillende knoppen tegelijk draaide.

Piet was vooral geïnteresseerd in het concept slaapkwaliteit en hij hamerde op het belang van de integratie van fysiologische en psychologische modellen. Onder Piet's leiding werd een slaapkwaliteitschaal ontwikkeld, een schaal die later in Groningen opnieuw gevalideerd zou worden en omgedoopt tot 'Groninger slaapkwaliteitschaal'.

Ook de internationale slaapwereld had zijn warme belangstelling. Toen de ESRS in 1980 een wanhopige oproep om hulp deed, omdat het congres door omstandigheden niet in Engeland kon plaatsvinden, bood Piet spontaan Amsterdam als locatie aan. Binnen een paar maanden

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PhD Theses

SLEEP IN LATER LIFE
A POPULATION-BASED APPROACH

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INTRODUCTION

Why do we sleep? How much sleep do we need? What happens if we do not sleep as much as we need, or indeed if we sleep too much? These questions have intrigued researchers for decades. Studies in rats have shown that sleep deprivation causes several physical changes, including increased food intake, weight loss, increased energy expenditure, decreased body temperature, and, after approximately two weeks, death. Although the significance of this syndrome for the function of sleep is not entirely clear, the main mechanism eventually leading to death appears to be that the rat's brain loses the power to regulate body temperature. The dramatic physical effects of sleep deprivation in rats and other small mammals have not been demonstrated in humans. Experiments in humans showed that the most prominent effects of prolonged sleep deprivation are apparent in the executive functions of the frontal lobe of the brain, including decreased vigilance, concentration, and reaction speed. Sleep deprivation also affects mood and increases irritability, and in some people, it may cause distorted perception and even hallucinations. In addition, laboratory studies have shown that sleep restriction also leads to an altered glucose metabolism and to lower circulating levels of leptin and higher levels of ghrelin. Leptin is a hormone that decreases appetite, while ghrelin stimulates it; therefore, the hormonal changes that result from sleep deprivation cause an upregulation of appetite. In addition to these experiments, epidemiological studies have shown that the amount of sleep a person usually obtains may have long-term effects on their health. In 1964, Hammond investigated a large number of health-related variables and their associations with mortality in a study of 1,064,004 persons. Self-reported sleep duration was one of the variables recorded; Hammond reported that men sleeping about 7 hours per night had lower death rates than men who slept either more or less than this. This finding has often been replicated afterwards, although the precise mechanism, or mechanisms, behind this U-shaped association of habitual sleep duration with mortality is still under investigation.

Measuring habitual sleep patterns on a population level is not straightforward. In large studies, mostly self-report measures of sleep parameters are used, varying from one question in a telephone survey to elaborate sleep diaries. However, self-reported measures of sleep can be distorted by matters of perception. Particularly in insomniacs, misperception of sleep duration is common. This becomes clear when self-report measures are compared with objective measurements. The most accurate method to distinguish sleep from waking is polysomnography. This is an invasive and time-consuming method; and therefore is not feasible in large studies. An alternative method is actigraphy, a method that infers sleep and wakefulness from the presence or absence of arm movement. Actigraphy is not without its own shortcomings, but it is a reasonable alternative for polysomnography in large studies.

SCOPE OF THE THESIS

The objective of this thesis was twofold. Firstly, its aim was to investigate methods of assessing habitual sleep in population-based studies. The focus was mainly on actigraphy (Part I). Its second aim was to gain insight into the relationships of sleep duration with both cardiovascular risk factors (Part II) and psychiatric disorders (Part III). The research was conducted within the setting of the Rotterdam Study, a large prospective cohort study of community-dwelling inhabitants of a district of Rotterdam, aged 55 and over. A total of 1076 persons participated in an additional actigraphy study: they wore an actigraph and kept a sleep diary for, on average, six consecutive nights. The research described in chapters 1 through 5 is mainly based on this subsample of the Rotterdam Study population.

PART I: MEASURING SLEEP. METHODOLOGY AND GENDER DIFFERENCES

In **chapter 1** of this thesis, a study is described in which we compared two different methods of assessing Total Sleep Time (TST): actigraphy and sleep diaries. We calculated the level of disagreement with regard to TST between the results of the two measurement methods. In 34 % of the participants, the estimated TST in the sleep diaries deviated more than 1 hour from actigraphically measured TST. Poor sleep quality, as measured by actigraphic and subjective measures, was consistently associated with a high level of disagreement between assessment methods, albeit in opposite directions. Poor actigraphically measured sleep quality was often accompanied by longer subjective estimates of TST, whereas subjectively poor sleepers tended to report shorter TSTs in their diaries than were measured with actigraphy. Gender, age, bed time, get up time, depressive symptoms, cognitive function and functional disability were also associated with either level or direction of disagreement between subjective and actigraphic measures of TST. This phenomenon may bias the results of epidemiological studies into the (medical) consequences or correlates of sleep duration, as the determinants of disagreement are likely to be associated with many of these possible outcome measures.
Chapter 2 illustrates discrepancies between measurement methods from another perspective. In this study, gender differences in subjective and actigraphic sleep parameters were investigated. Women reported shorter TST, and less favorable sleep onset latency, sleep efficiency (TST divided by the time spent in bed) and global sleep quality than men. When assessed with actigraphy, however, women were found to have longer and less fragmented sleep than men; only actigraphic sleep onset latency did not differ between men and women. Gender differences in self-reported sleep parameters were partly explained by depressive symptoms and sleep medication use, which are both more common in women and related to poor self-reported sleep quality. The shorter actigraphically measured TST in men was partly explained by their higher alcohol consumption. However, none of the gender differences in self-reported or actigraphic sleep measures could be fully explained by adjustment for multiple covariates.

PART II: SLEEP AND CARDIOVASCULAR RISK FACTORS

In **chapter 3**, the association of sleep measures with body mass index (BMI) and obesity is investigated. We found a marked U-shaped association of actigraphically measured TST with BMI and obesity: both short sleep and long sleep were related to a higher BMI and a higher prevalence of obesity. Sleep fragmentation also increased the likelihood of a higher BMI and obesity. The relationship between short sleep and obesity was attenuated after adjustment for

sleep fragmentation, whereas the higher risk for long sleepers remained unchanged. However, a quadratic relationship between TST and BMI still existed after adjustment for sleep fragmentation. Exclusion of participants with probable sleep apnea only marginally changed these associations. Self-reported TST was not associated with BMI or obesity. Our cross-sectional design prevented us from gaining insight into possible temporal or causal relations. For this reason, we cannot rule out that obesity leads to a shorter or longer sleep duration, or to more fragmented sleep.

Chapter 4 concentrates on the possible relationship between TST and hypertension, as several large studies have shown that both short and long habitual TSTs increase the risk of hypertension in adults. This cross-sectional study was conducted with self-report measures of TST in 5058 participants of the Rotterdam Study, and with actigraphic measures in the actigraphy study subgroup. After adjustment for age and gender and additionally for BMI, smoking, depressive symptoms, sleep medication use, diabetes mellitus, myocardial infarction and stroke, no significant association was apparent between TST, whether measured by self-report or actigraphy, and blood pressure or hypertension.

In the study reported in **chapter 5**, we investigated whether objectively measured TST, time in bed and sleep fragmentation were associated with total cholesterol and high density lipoprotein (HDL) cholesterol level. This study was performed in 768 persons who did not use cholesterol lowering medication. We found that longer TST was related to a higher total cholesterol level and a higher total/HDL cholesterol ratio (a less favorable lipid profile). A longer TST was strongly related to a longer time in bed. The association of long TST with cholesterol that we found seems to reflect two different mechanisms, depending on the age of the participants. Our analyses showed that the relationship between TST and cholesterol was driven by the strong association between a longer time in bed and a higher total cholesterol level in the youngest age group of people under 65. However, in persons aged 70 or older, the association between TST and cholesterol seemed to be explained by sleep fragmentation, which was related to a lower total cholesterol level. This association could well be due to underlying illness.

PART III: SLEEP AND PSYCHIATRIC DISORDERS

The study described in **chapter 6** included 5019 participants of the Rotterdam Study. In these persons, self-reported TST and other sleep parameters were examined in relation to depressive disorders and anxiety disorders. We found that the average TST in elderly persons with one of these disorders did not differ from the average TST of those without these disorders. Rather, both short and long sleepers were more likely to be depressed or to have an anxiety disorder than persons with a TST of 7 - 8 hours. These associations were stronger in people who did not use psychoactive medication. Persons with a depressive disorder spent more time in bed than non-depressed persons. Finally, participants with a depressive disorder and a comorbid anxiety disorder reported a substantially shorter TST than persons without these disorders or with one of these disorders. The above indicates that in studies of sleep duration and health, psychiatric disorders should be carefully taken into account. They may be important confounders, precursors or intermediates of the relations under study.

GENERAL CONCLUSIONS

To date, no measure has been invented to assess habitual sleep patterns with perfect accuracy. Results obtained with different assessment methods can be biased in different

ways. With these considerations in mind, the best way to deal with this problem is to use as many measures of sleep as possible. A validated questionnaire can be used to assess subjective sleep quality, a sleep diary or, whenever possible, actigraphy to assess sleep patterns over multiple nights, and preferably one night of PSG to ascertain sleep apnea and sleep disorders such as narcolepsy or restless legs syndrome. If the results of association studies are consistent over the different measurement methods, this increases the credibility of the results. Except for the night of PSG, our studies met these criteria, and therefore we had excellent data for our research on the associations between sleep and cardiovascular risk factors. Briefly, we found that both short and long sleep were associated with a higher BMI and the prevalence of obesity, and that there was no association between sleep duration and hypertension. Furthermore, a longer sleep duration was related to a higher total cholesterol level and a higher total/HDL cholesterol ratio (a less favorable lipid profile), with different underlying mechanisms in different age groups. Finally, we reported that both long and short sleep were related to depressive disorders and anxiety disorders in a complex way, but this study was based on self-report measures only. Future longitudinal studies with a long follow-up period are needed to shed more light on the temporal associations between sleep parameters and health. All of these investigations will contribute to the final answer to the question with which this thesis started: why do we sleep?

PUBLICATIONS

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commentary on the dissertation by Julia van den Berg

SLEEP IN LATER LIFE

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Julia van den Berg received her Ph.D. degree based on a dissertation 'Sleep in later life' [1], which she defended at the Erasmus University Rotterdam on December 4 2008. Her promoter was Prof. Dr. Bert Hofman, with co-promoters Dr. Henning Tiemeier and Dr. Arie Knuistingh Neven. The objective of the thesis was to assess the quality of sleep in a population of elderly people and to obtain insights into the association of sleep measures with various cardiovascular parameters. She also studied sleep parameters in relation to psychiatric disorders such as depression and anxiety. Studies were carried out in the framework of the Rotterdam Study, ERGO ('Erasmus Rotterdam Gezondheids Onderzoek'), which was carried out among elder inhabitants of the Rotterdam quarter, Ommoord.

Sleep, in particular total sleep time, was assessed in more than thousand people, all aged 55 or over. Two sleep assessing methods were used: actigraphy, in which an actigraph measures physical body movements and sleep is inferred by a low activity level, and by a sleep diary (the Pittsburgh Sleep Quality Index), assessing a subjective measure of sleep. For a long time it has been known that there is an acceptable correspondence between objective and subjective measures when sleep is healthy, but that considerable deviations can occur in poor sleep. Mrs. van den Berg has also found these discrepancies. Moreover, she found two types of differences: when sleep is regarded as poor by actigraphic methods, it is often accompanied by a longer subjective duration, and when sleep is regarded as poor by the sleep diary, it tends to be accompanied by a longer objectively measured sleep. This is a new finding which, however, has not been further investigated nor even discussed. Julia only notes that this interesting phenomenon can cloud the interpretation of her epidemiological studies. Gender differences in subjective and objective established sleep are also well known, and van den Berg describes that women report a less favourable sleep than men, while they objectively have a better sleep. These gender differences cannot be explained in a simple way, but I have a strong feeling that gender differences are also related to the discrepancies between objectively measured and subjectively assessed sleep.

The U-shaped curve between sleep duration and mortality, as already long ago was described by Kripke and colleagues [2], shows that both short (< 6 hours per night) and long (> 8 hours per night) sleep are associated with a higher rate of mortality. In the light of the restorative function of sleep it is understandable that short sleep duration is associated with a higher mortality, but the association of a long sleep with a higher mortality is barely understood. It is often stated that these data are spoiled by the long time spent in bed by weak and ill people, which influences the mortality rate of genuine long sleepers. Inspired by this intriguing relationship and in an attempt to clear up this association, Julia van den Berg started with an epidemiological study towards sleep duration and cardiovascular disorders. She began to study the relationship of sleep length and elevated body mass index and obesity. In that study, a U-shaped relation was also found: both short and long sleep duration, as

measured by actigraphy, are associated with a higher body mass index and a higher prevalence of obesity. To explain the higher weights of short sleepers, sleep fragmentation seem to explain a major part of variation, though the direction of the causal relationship is still unknown. On the other hand, the association between long sleep and obesity is more debatable. It is not consistently found in literature, and, although statistically significant – if I have understood all statistical manipulations correctly –, it is a marginal result with a large variation. Moreover, this relationship is far less understood, and fragmentation and sleep apnoea play only a minor role. It is striking that this relationship is not found using subjective sleep assessment methods. The next study was the association between average sleep duration and hypertension, another important cardiovascular risk factor. Van den Berg's results strongly suggest that sleep duration, both assessed with actigraphy and self-report, is not associated with blood pressure and hypertension in the elderly. In contrast to this finding, it appeared that a higher total level of cholesterol and a higher level of high density lipoprotein cholesterol is associated with a longer sleep duration, assessed by actigraphy. Two separate mechanisms, a longer time in bed and sleep fragmentation, seem to explain this relationship. A remarkable point is that the association between cholesterol level and sleep duration is absent or weak when sleep duration is self-reported.

The last part of the dissertation is devoted to the role of sleep in depression and anxiety. Common in these psychiatric disorders is sleep disturbance, but the relationship of depression and anxiety with sleep duration is unclear. In this study, also embedded in the Rotterdam Study, sleep duration was only established with the Pittsburgh Sleep Quality Index and more than 5000 elderly persons were interviewed. Both long and short sleepers are more likely to have a depressive or an anxiety disorder than persons with average sleep duration of 7 or 7.5 hours. It also appeared that sleep parameters and psychiatric disorders are interrelated in a complex way and future research is necessary to disentangle these phenomena. This part of the dissertation does not stick well to the heart of the thesis, being the relation between sleep parameters and cardiovascular risk factors. Sleep in this psychiatric part is only assessed in a subjective way, and given the problems in the interpretation of subjective versus objective sleep parameters, outcomes do not add much to existing knowledge. Instead, it is a pity that these subjective-objective differences are not given more attention. For me this is more than a methodological problem, it is a fundamental problem in sleep research and the dissertation of Julia van den Berg has, in a way, put the finger on this sensitive spot. A theoretical framework to touch this problem, however, seems necessary. That does not alter the fact that the thesis of Julia van den Berg delivers a payload of valuable epidemiological insights into the relationship between sleep duration and various cardiovascular risk factors. In particular, the study throws light on the U-shaped association between sleep duration and mortality, although it is striking that only increased body weight and cholesterol level, and not enhanced blood pressure, explains parts of the complex association between sleep parameters and mortality.

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END-STAGE RENAL DISEASE: SLEEP DISTURBANCES AND THE BIOLOGICAL CLOCK

Birgit C.P. Koch
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INTRODUCTION

The focus of sleep studies in End-Stage Renal Disease (ESRD) has been on sleep apnea syndrome, restless legs and periodic limb movement disorder [1,2,3]. The focus of research on circadian rhythm in ESRD has been on the impaired diurnal blood pressure pattern. This impaired diurnal pattern is a risk factor for the development of cardiovascular disease [4,5].

In this thesis, we elucidated the possible external and internal negative factors involved in sleep-wake rhythmicity in ESRD patients. Endogenous melatonin profiles in renal disease have become a field of interest recently, although literature is still scarce and contradicting. All authors found an absence of the nocturnal melatonin rise in serum [6-8], but the melatonin levels in serum during daytime were increased [6] or decreased [8]. Sleep apnea is often seen in ESRD patients [9] and has been related to increased daytime melatonin levels [10]. These increased levels may be caused by pineal dysfynction in obstructive sleep apnea syndrome [10].

Decreased melatonin concentrations during daytime are more likely to be found in patients with renal failure, because of increased levels of blood urea nitrogen. These increased levels are associated with adrenoreceptor downregulation [11]. Adrenoreceptors are important in the pathway of melatonin synthesis [12].

Several approaches can resynchronize the rhythm: bright light, exogenous erythropoietin [7], exogenous melatonin, cool dialysate [13], exercise and nocturnal dialysis. Most approaches have been researched in other patient groups.
The main objectives of the thesis were:

- Is the circadian sleep-wake rhythm in patients with ESRD impaired?
- Does the rhythm of endogenous melatonin, as a synchronizer of the sleep-wake rhythm, decrease with advancing renal dysfunction?
- What external or internal factors or approaches can affect the sleep-wake pattern in the ESRD population?

PREVALENCE

Subjective sleep efficiency, hypnotics and potentially sleep-disturbing medication in hemodialysis patients

We found that subjective sleep efficiency in our patient group was significantly impaired in comparison to the control group [14]. In addition, the classical hypnotics were not successfully used. This lack of efficacy of hypnotics has been reported earlier in the general older population [15]. Surprisingly, no association was found between dialysis adequacy and sleep efficiency. We expected to find this association, because of the described relationships between urea, melatonin and sleep apnea [2,3,11]. An explanation could be that standard dialysis adequacy focuses on the clearance of the small molecule urea, and that sleep might be related to the clearance of middle-sized or larger molecules. As we did not find the

standard age or gender effect in the prevalence of sleep disturbances, we hypothesized that the pathophysiology of sleep disturbances may differ in the medically ill in comparison to the general population. This hypothesis has been questioned earlier in literature [16]. Our hemodialysis population used significantly more benzodiazepines and beta-blockers, in comparison to the matched general population [17]. Due to the fact that beta-blockers disturb melatonin rhythm and increase nightmares, clinicians should be aware of existent sleep disturbances when prescribing lipophilic betablockers in this patient group.

Actigraphy measures in hemodialysis patients

Actigraphy has become a valid alternative in case polysomnography is not feasible in insomnia patients [18]. We found that actigraphy can be valuable in evaluating sleep-wake rhythm disturbances and timing of sleep in the ESRD population [19]. Hemodialysis patients expressed more impaired sleep parameters in comparison to insomnia patients. We concluded that in hemodialysis patients in whom sleep apneas and PLMD are not suspected, actigraphy can be used as a useful tool to objectify sleep disturbances, in addition to sleep questionnaires.

Sleep-wake rhythms and endogenous melatonin rhythm in nocturnal hemodialysis, daytime hemodialysis and automated peritoneal dialysis patients

Using actigraphy, we found that daytime hemodialysis patients experienced the worst sleep, in comparison to nocturnal hemodialysis and automated peritoneal dialysis patients [20]. With respect to sleep-wake rhythm, nocturnal hemodialysis has advantages, compared to daytime dialysis. The sleep promoting effects of dialysis coincide with the appropriate and conventional time of day. In this way, the shift to nocturnal dialysis could restore the normal temporal relationship between the sleep period and the other rhythms of the circadian system. The increased clearance contributes to this advantage of nocturnal hemodialysis. Automated peritoneal dialysis, which is performed during night time, resulted in more disturbed melatonin rhythm than nocturnal hemodialysis [20]. The timing of dialysis seemed not to affect the melatonin rhythm. This observation, probably due to more autonomic deregulation in peritoneal dialysis [21] or the higher toxin clearance in nocturnal hemodialysis [3,22], can indicate the superior effect of elevated clearance above timing of day in nocturnal dialysis with regards to melatonin rhythm. Peritoneal dialysis patients did not sleep worse than nocturnal hemodialysis patients. This suggests that the melatonin rhythm might have a less significant role in sleep-wake rhythm of peritoneal dialysis patients, compared to hemodialysis patients.

CIRCADIAN RHYTHMS IN CHRONIC KIDNEY DISEASE

Although this thesis concerns mostly ESRD, circadian rhythm was researched also in chronic kidney disease to research if circadian rhythm is already disturbed prior to dialysis treatment.

Circadian rhythms of melatonin, cortisol and core body temperature

The main result of our study is that renal function is associated with melatonin rhythm, but not with cortisol or core body temperature rhythm [23]. As endogenous melatonin rhythm was more impaired with advanced renal dysfunction, a future role for exogenous melatonin in improving endogenous melatonin rhythm, and subsequently sleep of patients with chronic kidney disease can be exerted. Lowered endogenous melatonin levels have been associated with increased oxidative stress [24,25] and impaired immune response [26]. In addition, exogenous melatonin has been useful in restoring the nocturnal dipping profile in male patients with essential hypertension [27]. As patients with chronic kidney disease often

exhibit a non-dipper profile [4,5], restoring circadian blood pressure rhythm in this population by means of exogenous melatonin may be a future research topic.

While melatonin has been proposed to be an endogenous synchronizer [28], able to stabilize other circadian rhythms under normal circumstances, we failed to find an association between melatonin and core body temperature in patients with chronic kidney disease [24]. Due to the impaired melatonin rhythm, melatonin might not have the role of endogenous synchronizator, and cannot affect core body temperature, which does occur under normal circumstances [29]. Due to frequently-observed sleep-wake disturbances in patients with chronic kidney disease, affected cortisol rhythms might have been expected [30]. Nevertheless, in most patients we found a normal cortisol rhythm, not dependent on renal function. Even though melatonin rhythm was impaired in patients with chronic kidney disease, dialysis patients still experienced the most deteriorated melatonin production [20,24], suggesting an additional contribution of the dialysis process, besides renal dysfunction, to melatonin rhythm.

Circadian rhythms of erythropoietin and insulin growth factor

Conflicting data are found in literature, on whether a circadian rhythm of erythropoietin (EPO) and Insulin Growth Factor (IGF-1) exists. Nevertheless, appreciating the physiological rhythm can have clinical consequences for the treatment of anaemia. Although we found a rhythm for EPO, the peak times varied [31]. Therefore, it is unlikely that EPO levels are regulated in a circadian fashion. Importantly, no relationship was found between the presence of an EPO rhythm and the degree of chronic kidney disease [31]. It thus remains unclear why subjects present an EPO rhythm. A possible cause could be that in the subjects with an EPO rhythm, more fluctuations in blood pressure are present [32]. Another cause can be that subjects have obstructive sleep apnea syndrome (OSAS) that had not been diagnosed. In OSAS, diurnal variation in EPO levels is higher than in healthy persons, due to nocturnal hypoxemia [33].

Furthermore, we were unable to demonstrate a circadian rhythm for IGF-1 in subjects with a decreased renal function, but we also did not find a IGF-1 rhythm in subjects with a normal renal function. A possible explanation is that IGF-1 rhythmicity indeed is absent in adults both with and without CKD, but present in children [34]. Another explanation for the absence of an IGF-1 rhythm in our study could be the relatively high prevalence of Diabetes Mellitus (DM) amongst the subjects, as DM is associated with lower levels of IGF-1 [35].

An interesting finding of this study is that mean levels of IGF-1 were correlated inversely to mean levels of EPO [31]. Subjects with the greatest decrease in renal function and a relatively low haemoglobin had the highest IGF-1 levels. This result suggests that EPO and IGF-1 both have a role in erythropoiesis in chronic kidney disease. All together, IGF-1 may constitute a potent pro-erythropoietic agent in chronic kidney disease. However, until now its therapeutic application is limited [36].

INTERVENTION STUDIES

Nocturnal dialysis

Nocturnal hemodialysis resulted in significant improvements of sleep parameters, measured with polysomnography in comparison to daytime hemodialysis [37]. Subjective measured sleep was also less impaired with nocturnal hemodialysis, compared to daytime hemodialysis. Sleep apnea tended to decrease. This decrease has also been found, with even

more impressive results, in patients who received nocturnal hemodialysis at home [2]. Nocturnal hemodialysis at home is performed 7 nights a week, while nocturnal in-hospital hemodialysis is carried out only 4 nights a week. The difference in sleep apnea reduction between in-hospital or at home nocturnal hemodialysis may, therefore, be found in the more frequent dialysis, subsequently the intensified fluid clearance.

The nocturnal melatonin surge was partially recovered after the intervention of nocturnal hemodialysis, probably due to both increased urea clearance and time of dialysis treatment [37]. Research on long daytime dialysis [22] has not been focused on circadian rhythm and sleep. When research on this theme is performed in the future, it can discriminate if longer dialysis or the time of day is the most prominent factor in melatonin rhythm return and improved sleep in nocturnal hemodialysis.

Exogenous melatonin

Exogenous melatonin led to an improvement of sleep parameters, when compared to placebo, in daytime hemodialysis patients [38]. In addition, the nocturnal melatonin rise was recovered. The recovery was more distinct when compared to nocturnal hemodialysis. This result suggests that exogenous melatonin might have a larger role than time of day or toxin clearance in improving melatonin and sleep-wake rhythm. The promising results of exogenous melatonin can indicate that the biological clock is a more prominent factor, compared to dialysis clearance. Under normal circumstances, exogenous melatonin causes phase-resetting of the melatonin rhythm [39]. In this study, however, with absent endogenous melatonin rhythm, exogenous melatonin might exert another role, constituting a masking factor that selectively affects the onset of the melatonin curve. After a period of melatonin administration, the activity of the enzymes, involved in melatonin synthesis, may have been sensitized. That would facilitate the triggering of the synthesis and release of endogenous melatonin [40]. Most melatonin studies are performed in small patient groups during short investigation periods. Due to this flaw in research, a study on MELatOnin anD qualitY of life (MELODY study) was recently started. This is a long-term (1 year) placebo-controlled study on the effects of melatonin 3 mg on sleep, quality of life and cardiovascular outcomes, to confirm the role of exogenous melatonin in ESRD. The last patient (n=70) has just been included.

In summary, when returning to the questions presented in the introduction of this thesis, the main objective: circadian sleep-wake rhythm in patients with ESRD is indeed impaired. Although the internal factors have a prominent impact on sleep disturbances, the external factors contribute to these disturbances: - Endogenous melatonin rhythm decreased with advancing renal dysfunction. The dialysis process has an additional negative contribution to the impaired rhythm in hemodialysis patients, when comparing melatonin rhythms of hemodialysis and pre-dialysis patients-. Under normal circumstances, endogenous melatonin exerts its role as endogenous synchronizer, thereby phase-shifting circadian rhythm of core body temperature. Caused by the abolished melatonin rhythm, melatonin might not exert this role in ESRD. The presence of the circadian rhythms of temperature, erythropoietin and cortisol is not associated with renal function. Interesting findings on exogenous melatonin are presented. In future, more research is warranted on the other approaches to establish which intervention has to be followed in the dialysis patient with sleep disturbances.

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commentary on the dissertation by Birgit Koch

CIRCADIAN RHYTHMS IN PATIENTS WITH KIDNEY DISEASE

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Birgit Koch (1978) defended her doctorate thesis entitled 'End-stage renal disease: sleep disturbances and the biological clock' in Amsterdam (VU) on the nice rhythmical date of 09-09-2009. Her promotores were Professors Piet ter Wee and Gerard Kerkhof. Elsbeth Nagtegaal was co-promotor.

The purple cover of the thesis shows a pink stylized brain-kidney melting with a clock in between. This is the essence of the thesis: the influence of the biological clock in several circadian rhythms in the kidney. As Birgit tells at the end of the thesis the basic idea to investigate sleep wake rhythm in patients with end-stage renal disease was born when two Dutch nephrologists were waiting on their plane back after a renal congress in the USA.
The idea fell on fertile soil with knowledge of circadian rhythms and chronobiological effects of melatonin in the clinical pharmacy, strong interest in sleep problems in the sleepcenter and a progressive and patient oriented department for haemodialysis in the Meander Medical Center. At that moment the department for haemodialysis was busy to initiate nocturnal–in–hospital haemodialysis. In the summer of 2004, Meander Medical Center was the first Dutch hospital with the possibility to dialyse in-hospital at night. All these building stones together were the tasteful ingredients for the interesting and clinical oriented thesis of Birgit Koch.

In the Introduction it is clearly explained which field of sleep studies in patients with end stage renal disease has been studied until now. Disturbances in circadian rhythms cannot only affect sleep, but also have a prominent role in the pathology of renal disease. This is an area which has not received the attention it deserves. In the second part, about prevalence, subjective sleep efficiency, prescription of hypnotics and potentially sleep-disturbing medication, actigraphy, melatonin rhythm and sleep wake rhythms in hemodialysis patients are described. It becomes clear that subjective sleep efficiency was significantly impaired in comparison to a control group. In addition the classical hypnotics were not successfully used.

All these findings justified the intervention studies described in the last part. In this third part the circadian rhythm in chronic kidney disease patients is studied. The objectives were, primary, to investigate whether the circadian rhythm is already disturbed prior to dialysis treatment. Second when comparing the melatonin data of dialysis patients and patients with chronic kidney disease the additional negative contribution of dialysis could be estimated. The answers obtained from these studies described in this part are that with a decline in kidney function the melatonin rhythm diminishes, however, dialysis patients still experience the most deteriorated melatonin production, suggesting a contribution of the dialysis process besides renal dysfunction to melatonin rhythm.

No relationship was found between the presence of erythropoietin and the degree of chronic kidney disease, neither a circadian rhythm for Insuline-like Growth Factor-1 (IGF-1) in subjects with a decreased renal function. An interesting finding, however, was the inverse

correlation of the mean levels of IGF-1 and erythropoietin. This result suggests that erythropoietin and IGF-1 both have a role in erythropoiesis in chronic kidney disease: when the concentration of erythropoietin is not sufficient in resolving anaemia, levels of IGF-1 increase. All together, IGF-1 may constitute a potent pro-erythropoietic agent in chronic kidney disease. However, until now its therapeutic application is limited by the difficulty of assessing pituitary functional status in patients with chronic kidney disease and the interaction with IGF binding proteins that determine its bioavailability.

In the fourth part two intervention studies are described, to investigate the circadian sleep-wake rhythm: changing from daytime to nocturnal haemodialysis and providing exogenous melatonin. The impressive improvement of the patients undergoing nocturnal dialysis went together with the improvement of the melatonin rhythm, compared to the melatonin rhythm in day-hemodialysis patients. For the future it must be investigated whether longer dialysis or the time of day is the prominent factor in melatonin rhythm return and improved sleep in nocturnal haemodialysis. The other intervention study describes the effect of application of melatonin in a placebo controlled study in daytime dialysis patients. Exogenous melatonin led to an improvement of sleep parameters, when compared to placebo, in daytime hemodialysis patients. Sleep onset normalized and the nocturnal melatonin rise was recovered. This was more distinct when compared to nocturnal haemodialysis. This result suggests that exogenous melatonin might have a larger role than time of day or toxin clearance in improving melatonin and sleep-wake rhythm. The promising results of exogenous melatonin can indicate that the biological clock is a more prominent factor, compared to dialysis clearance, than suspected in advance.

This thesis offers a lot of useful information about the circadian rhythms in patients with end stage renal disease. More attention and awareness for serious sleep-wake problems, circadian rhythm disturbances and their effects on the quality of life in patients with end stage renal disease has been reached by the studies described in this thesis. The relation between ‘hard’ somatic disease and ‘soft’ inconveniences like a disturbed sleep-wake rhythm has become clearer. The fact that the study of Birgit Koch was granted by the Dutch Kidney Foundation is interesting and promising in this respect.

The interesting discussions with all people involved in these studies showed how important it is to share knowledge of the different disciplines which come together in the care and cure of end stage renal disease in general and the disturbances and opportunities to improve the circadian rhythms in this population.

For researchers and clinicians in the field of sleep, nephrology, physiology and pharmacology this thesis will provide a lot of interesting information and offers directions for future research into the relationships between circadian rhythm and kidney disease.

HYPNOTICS AND ANXIOLYTICS
FIELD AND LABORATORY MEASURES OF DRUG SAFETY IN DRIVING PERFORMANCE AND COGNITIVE FUNCTIONS

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One of the risk factors influencing crash involvement is the use of medicinal drugs and in particular the so-called 'psychoactive drugs'. Psychoactive medicinal drugs act primarily on the central nervous system and are widely used for the treatment of a variety of psychiatric and neurological problems. Among the most frequently prescribed psychoactive medicinal drugs are GABAergic anxiolytics and hypnotics, for the treatment of anxiety disorders and insomnia, respectively. Besides therapeutic effects, anxiolytics and hypnotics often produce side-effects or residual effects. They impair cognitive and psychomotor functions and negatively affect performance in a variety of tasks, such as driving.

To date, the impairing effects on driving of anxiolytics and hypnotics have been widely established in a large number of experimental studies mainly conducted with healthy young volunteers. Despite the vast amount of existing data concerning the effects of anxiolytics and hypnotics on driving, a number of questions still have remain unanswered. It is still not clear whether the results of experimental studies conducted with healthy young volunteers translate to therapeutic use in patients. Furthermore, it has not yet been clarified if residual effects of hypnotics are manifested differently between female and male users. To conclude, it has not been studied what the influence of change in formulation has on the adverse effects of an anxiolytic on driving performance.

Therefore, the aim of this dissertation is to evaluate to what extent the effects of anxiolytics and hypnotics on driving performance are modulated by factors, such as age, gender, disorder or drug formulation.

Chapter 1 - The study described in this chapter assessed the residual effects of evening and middle-of-night administration of gaboxadol 15 mg, evening administration of zopiclone 7.5 mg and middle-of-the-night administration of zolpidem 10mg, on cognitive, psychomotor and driving performance in healthy young volunteers. A total of 25 young volunteers (12 women; mean age 31.4 years) completed a double-blind, placebo-controlled, active-referenced five-way cross over study. Each treatment night subjects ingested one capsule at 23:00 hours and one at 04:00 hours. Treatments were placebo at both times, gaboxadol 15 mg or zopiclone 7.5 mg followed by placebo, and placebo followed by gaboxadol 15 mg or zolpidem 10 mg. Effects on cognition and psychomotor performance were assessed between 07:30-08:30 hours, and on driving between 09:00-10:00 hours. Driving performance after evening administration of gaboxadol 15 mg was not significantly impaired. Evening administration of zopiclone 7.5 mg and middle-of-the-night administration of gaboxadol 15 mg and zolpidem 10 mg resulted in significantly impaired driving performance. Evening administration of gaboxadol had minor effects on divided attention only, whereas middle-of-the-night administration significantly impaired performance in all tests except memory. Zolpidem and zopiclone significantly impaired performance in every test except tracking after zopiclone. Gaboxadol 15 mg can produce minor residual effects on driving after

evening administration. Administration later at night is associated with moderately impairing residual effects on driving and psychomotor performance, but not on memory.

Chapter 2 - A limitation of the study described in the former chapter was that residual effects were established in younger drivers. The majority of users of hypnotics are older people, however, who may be more sensitive to drug effects. The aim of this study was to evaluate the residual effects the morning after evening doses of temazepam 20 mg and zopiclone 7.5 mg on driving performance in healthy elderly drivers. Eighteen healthy elderly drivers (10 female and 8 male; mean age 64.3 years) participated in a double-blind, three-way crossover study. Treatments were single oral doses of temazepam 20 mg, zopiclone 7.5 mg and placebo administered at bedtime. Subjects performed a standardized highway driving test between 10 and 11 hours after hypnotic intake. Before and after the driving test cognitive performance was assessed. Driving performance did not differ between temazepam and placebo, but was significantly impaired following zopiclone 7.5 mg ($p<0.002$). The results of the laboratory tests were in line with the effects on driving of both hypnotics. It was concluded that temazepam 20 mg is unlikely to impair driving 10 hours or more after bedtime administration in healthy elderly aged 75 years or younger. Zopiclone 7.5 mg moderately impairs driving in elderly at least until 11 hours after administration. The magnitude of impairing effects in elderly was comparable to those found previously in younger volunteers.

Chapter 3 - In many European countries, Canada and Japan, the non-benzodiazepine zopiclone now is among the most frequently prescribed hypnotic drugs. This can be explained by the growing view among physician's that zopiclone is more effective and safer than conventional benzodiazepines. It has been shown, however, in four studies using similar procedures that zopiclone 7.5 mg produces moderate to severe impairment on driving performance. The study described in this chapter aimed to review these studies and analyze the pooled data to determine whether the severity of effects is modified by the gender and age of the subjects. Results showed that zopiclone 7.5 mg has significant and clinically relevant impairing effects on driving performance in the morning, until 11 hours after bedtime ingestion. The effects did not differ between males and females and did not increase with age, at least until 75 years. It was concluded that patients using an evening dose of zopiclone 7.5 mg should avoid activity in skilled work and participation in traffic the morning after intake. General practitioners' beliefs about the beneficial safety profile of zopiclone may need adjustment and patients using zopiclone 7.5 mg should be warned accordingly. In addition, there is no need to differentiate warnings about zopiclone's residual impairing effects depending on the gender of the patient.

Chapter 4 - It has not yet been clarified to what extent actual driving performance is affected by insomnia. In addition, it remains to be determined whether chronic use of hypnotics has deteriorating effects on driving performance in patients suffering from insomnia. Therefore, the aim of the present study was to explore the effects of insomnia and chronic use of hypnotics on driving in a one hour standardized driving test in actual traffic. A total of 22 elderly insomnia patients chronically using hypnotics, 20 elderly insomniacs infrequently or not using hypnotics and 21 healthy, age-matched controls performed a standardized highway driving test between 10 and 11 hours after bedtime. Before the driving test cognitive performance was assessed. Results indicate that driving performance is not impaired in patients suffering from insomnia, irrespective of use of hypnotics. In addition, driving related psychomotor and cognitive performance appeared not to be affected in medicated and unmedicated insomnia patients. Insomnia patients appear to be able to successfully perform a

one hour driving task that requires prolonged attentional demands. Chronic use of hypnotic does not seem to change driving performance.

Chapter 5 – As mentioned in the previous chapters, residual effects of hypnotics on actual driving performance have been mainly determined in studies using a standardized driving test with healthy good sleepers. Responses to these effects may differ, however, between insomniacs and healthy volunteers due to the underlying sleep disorder. Performance in insomniacs is expected to improve due to the sleep improving effects of hypnotics and may attenuate the impairing effects. In addition, a majority of insomniacs uses hypnotics chronically resulting in the development of tolerance to impairing effects. Impaired driving performance in healthy volunteers may then be an overestimation of the actual effects in insomniacs. The study described in this chapter aimed to compare the residual effects of the frequently prescribed hypnotic zopiclone 7.5 mg on driving performance of 16 elderly insomniacs chronically using hypnotics (frequent users), 16 elderly insomniacs not or infrequently using hypnotics (infrequent users) and 16 healthy, age matched, good sleepers (controls). The study was conducted according to a 3x2 double-blind, placebo controlled crossover design, with three groups and two treatment conditions. Treatments were single oral doses of zopiclone 7.5 mg and placebo administered immediately before retiring to bed at 23:30 hours. Between 10 and 11 hours after administration subjects performed a standardized highway driving test. Results indicated that zopiclone 7.5 mg significantly impaired driving performance in both insomnia groups and healthy controls. The magnitude of impairment was, however, significantly less in the frequent users group as compared with the controls. Effects found in the infrequent users were in line with previous studies, suggesting that these studies are able to validly predict the residual effects of hypnotics in insomnia patients who do not or infrequently use hypnotics. Chronic use of hypnotics seems to attenuate the severity of effects of zopiclone 7.5 mg. Nevertheless, this reduction does not result in an absence of impairing effects in insomnia patients chronically using hypnotics.

Chapter 6 - Alprazolam extended-release (XR) is approved for the treatment of panic disorder. This sustained formulation is absorbed in a delayed manner and is therefore expected to produce fewer and less severe side effects than its immediate release equivalent (alprazolam IR). The effect of alprazolam XR on potentially dangerous daily activities, such as driving a car, is expected to be less as compared to alprazolam IR. The study presented in this chapter was designed to compare the effects of alprazolam XR (1 mg) and alprazolam IR (1 mg) on actual driving ability and cognitive function. Eighteen healthy volunteers (aged 20-45 years) participated in a double blind, placebo-controlled, three-way crossover study. At 4 hours post dose, subjects performed a standardized driving test on a primary highway in normal traffic. Cognitive and psychomotor tests were assessed 1, 2.5 and 5.5 hours post dose. Memory functioning was measured only 1 hour after administration. Results showed that both formulations severely impaired driving performance between 4 and 5 hours after administration. The magnitude of impairment in the driving test observed with alprazolam XR was about half that observed with alprazolam IR. Laboratory test results were in line with the driving data. It was concluded that the acute impairing effects of alprazolam XR 1 mg on driving and psychomotor functions were generally less as compared to its immediate release equivalent, but still of sufficient magnitude to increase the risk of becoming involved in traffic accidents.

Chapter 7 – This chapter summarizes and discusses the results from the studies described in the previous chapters. Results showed that performance of the driving test after administration of hypnotics was not differentially affected by age, gender or insomnia. It was

therefore concluded that studies investigating the residual effects of hypnotics on driving performance in young, healthy volunteers validly predict the effects of hypnotics in elderly, insomnia patients. Lastly, the effects of an extended release formulation of a benzodiazepine anxiolytic on highway driving appeared to be significantly reduced when compared to its immediate release equivalent. Yet, it was concluded that the sedative effects of the extended release version were still of significant magnitude, resulting in an increased risk of becoming involved in a car accident.

commentary on the dissertation by Tim Leufkens

FACTORS MODIFYING DRUG INDUCED PERFORMANCE IMPAIRMENT

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Tim Leufkens (1978) defended his PhD thesis entitled "*Hypnotics and anxiolytics: field and laboratory measures of drug safety in driving performance and cognitive functions"* on 9 October 2009 at Maastricht University. Prof. dr. Wim J. Riedel acted as his promotor and Dr. Annemiek Vermeeren as his co-promotor. Tim Leufkens conducted his research as PhD student at the department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University. His work focused on factors that can modify the severity of performance impairment associated with the use of hypnotics or anxiolytics, such as age, gender, the underlying disease, development of tolerance and the formulation of the drug. The common method used is a standardized "highway driving test', which is a one-hour over-the-road driving test in normal traffic, developed and uniquely used in the Netherlands.

The first and last chapters on original research (chapters 1 and 6) concern studies designed to evaluate the sedative side effects and associated impairment of cognitive functions and driving performance of a newly developed hypnotic and a new formulation of a benzodiazepine anxiolytic in healthy young volunteers. In contrast to what was expected on the basis of the hypnotic's kinetics and results from clinical trials, the drug was found to have measurable residual effects on performance the next morning. Although, these results illustrate that the methods used in clinical trials may not have been sufficiently powerful to detect minor to moderately severe effects of drugs on performance, it might also be argued that effects in patients differ from those in healthy volunteers, for example due to the interactions with the underlying disease. (This issue is taken up by Leufkens in chapter 4 and 5.) The study described in chapter 1 has recently been published in the Journal of Sleep Research (2009, on line since June), and the study in chapter 6 was already published in Psychopharmacology (2007, 191: 951-959).

As most users of hypnotics are older and female, the questions were raised whether elderly or female drivers may be more sensitive to residual effects of hypnotics. The study described in chapter 2 (J Clin Pharmacol 2009, 5: in press) shows that zopiclone 7.5 mg taken at bedtime significantly impaired next day driving performance in elderly drivers, whereas temazepam 20 mg did not. The latter seems to be good news for many patients, as temazepam is the most frequently prescribed hypnotic in most European countries. Zopiclone's effects on driving are reviewed in chapter 3, where data from four separate driving studies conducted by Leufkens and Vermeeren are pooled and re-analyzed. Although zopiclone is generally considered safe by clinicians with respect to its potential to produce residual effects due to its short half-life (5 hours), results of pooled analysis clearly show that the 7.5 mg dose impairs driving between 10 and 11 hours after bedtime administration. Effects are on average comparable to or more severe than those produced by alcohol in the same test when subjects drive with blood alcohol concentrations of 0.5 mg/ml, i.e. the legal limit for alcohol when

driving in most countries. No differences in sensitivity to these effects were found between males and females, and between young and elderly drivers.

The studies described in chapters 4 and 5 are particularly important for clinical practice. It is often argued that effects in healthy volunteers differ from those in patients, due to effects of the underlying disease and changes in drug effects with long term use. So far there are hardly any studies published, however, that assessed the effects of insomnia or long term use of hypnotics on driving, or that directly compare the residual effects of a hypnotic between patients and healthy volunteers. The studies described in chapter 4 and 5 are, as far as I know, the first to test these hypotheses. Both studies were conducted as part of a large European project entitled "Driving Under Influence of Drugs, Alcohol and Medicines (DRUID; www.druid-project.eu)' and financed by the European Community within the framework of the EU 6th Framework Program. Polysomnographic data in these studies were recorded and analyzed in collaboration with the group of Al de Weerd in Zwolle.

The study described in chapter 4 showed that driving performance of patients complaining of insomnia did not differ from that of normal sleepers of the same age. Furthermore it was found that driving performance of patients chronically using hypnotics did not differ from that of normal sleepers. The latter finding might suggest that patients had developed tolerance to the residual effects, but it might also be explained by the specific drugs and doses used by these patients. Most patients in this study used drugs and doses of which it is known that they are unlikely to produce residual effects, which may indicate that prescribing physicians are aware of the risks associated with residual effects of hypnotics. It could be argued that safety was achieved at the expense of efficacy as the chronic users still reported to sleep worse than controls, despite using a hypnotic. The study described in chapter 5 was designed as a double blind, placebo controlled, crossover study comparing the residual effects of zopiclone 7.5 mg in untreated insomnia patients, chronic users of hypnotics and controls. Results showed that, similar to controls, driving performance of insomnia patients and chronic users was significantly impaired the morning after bedtime use of zopiclone. So despite the therapeutic effects on sleep and development of tolerance, zopiclone 7.5 mg still impaired next day's driving in patients. This illustrates that patients should still be careful when driving after use of drugs and doses known to produce residual effects in healthy volunteers, but also that there is responsibility for prescribing physicians to consider the risk of residual sedation for patients who drive. More information on this topic can be found in Tim Leufkens' dissertation and in the publications that will undoubtedly follow from these studies, as Tim collected many more data that still have to be analyzed.

SLEEP-WAKE
Research in The Netherlands

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Mini review

VALIUM
STERNBACH'S GOLDEN BENZODIAZEPINE STORY

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PROLOGUE

Exactly 50 years ago, on July 7 1959 US patent nr. 2,893,992 was granted to Leo Henryk Sternbach for the drug chloordiazepoxide, the first benzodiazepine [1]. This drug entered the market under the trade name Librium in 1960. Soon after, in 1963, this benzodiazepine was followed by diazepam, much better known under the trade mark Valium, a fantasy name related to concepts as 'value' and 'valid'. This drug became a goldmine for the pharmaceutical company Hoffmann-La Roche at Basel (Switzerland) and Nutley, New Jersey, (USA). It was the beginning of a success story, given that benzodiazepines are the most prescribed pharmaceutics in the world. They are used as anxiolytics, hypnotics, anticonvulsants, muscle relaxants, and as mood stabilizers. Valium is still the most well-known drug of the large benzodiazepine family. It got the image that it is a panacea for everything: 'Wie mann nicht weisst, wie was und warum, dann gibt man immer Valium'.

LEO HENRYK STERNBACH

On May 7 1908, approximately 100 years ago, Leo Henryk Sternbach [2] (Figure 1) was born in Abbazia, at that time an Adriatic resort town in the Austro-Hungarian empire, and now known as Opatija, a city on the Istrian peninsula of the republic of Croatia. Sternbach was the elder son of a Polish Jew and his Hungarian Jewish wife, and his mother tongue was Hungarian and German. When the pharmacy of father Michael Sternbach fell into a crisis caused by political changes in Abbazia, which came under control of Italy after World War I, the family moved in 1926 to Kraków in the newly formed republic of Poland, birth place of father Sternbach. There he opened a pharmacy in the Jewish quarter Kazimierz.

In 1926, young Leo, graduated from a German secondary school in Bielsko-Biała in the south of Poland, then moved to Kraków, where he enrolled at the Jagiellonian University, starting with a study in pharmacy. In 1929 he was awarded a master's degree in pharmacy and immediately began a second study, now of organic chemistry, under professor Karol Dziewoński. He finished this study in 1931. From 1931 until 1936 he held a position as a research assistant and lecturer in the team of Dziewoński. In this period Sternbach was looking for new dyes and came across products known as benzo-heptoxdiazines [3]. These were rather interesting drugs due to their biologically active properties. However, in that time, when Sternbach's job was to search for useful dyes, the research on these benzo-heptoxdiazines, the cradle of the benzodiazepines, was abandoned.

Growing anti-Semitism was the reason that Dziewoński was not longer able to keep his Jewish assistant. He obtained a scholarship for him and Sternbach moved to Vienna, where, unfortunately, the situation was no better than in Kraków. Therefore, he soon left Vienna for Zurich in Switzerland, where he arrived in the autumn of 1937. A job was offered to him at the ETH, the Federal Institute of Technology. There he collaborated with the famous 1939

chemistry Nobel prize winner, Leopold Ruzicka. Sternbach got living accommodations at a certain Mrs. Kreuzer, where he met her daughter, Herta. He married Herta Kreuzer in 1940 who remained his wife till Sternbach's death in 2005 and the mother of his two sons, Michael and Daniel.
The ETH was not free from racially motivated problems caused by World War II, thus Sternbach, knowing that Hoffmann-La Roche was looking for a research scientist, applied for this post. On May 7 1940, on his 32nd birthday, he was offered this job at Roche. Optimistic as always, Sternbach started to work on the synthesis of vitamin B2, but impending personnel changes caused by the war were not far away. With the collapse of Belgium and France the war approached Switzerland, and Roche took the decision to move its headquarters from Basel to Nutley, New Jersey, in the USA. Moreover, the company assisted in the flight of a group of Jewish scientists, including Sternbach and his wife, to the USA. After a dangerous trip through Western Europe, a ship, the Serpa Pinta, left Lisbon (Portugal) on June 12 1941, arriving in Jersey City (USA) on June 22 1941. After German, Hungarian, and Polish, Sternbach was travelling to his fourth language, American English. In the beginning Sternbach was not very happy nor successful with his early work at Roche. It took time to build a group of like-minded researchers around him and to find challenging research. He found it in the synthesis of biotine, a vitamin of the B complexes, and in the synthesis of the bleeding blocker trimethaphan (Arfonad). This propelled Sternbach to the select group of top researchers at Roche. In the mean time the Sternbach-couple moved from their rented accommodations in Upper Montclair (New Jersey) to their own home in the same place. Later, in 2003, they moved to Chapel Hill (North Carolina).

SYNTHESIS OF BENZODIAZEPINES

In the mid-fifties Roche developed a program that focused on tranquilizers (anxiolytics), a new group of preparations of potentially high clinical importance. Sternbach recalled a few substances that he had discovered 20 years earlier with Dziewoński at the Jagiellonian University. He started with these benzo-heptoxdiazines, but lost his way in the synthesis of the substances he had in mind. Unfortunately, he even ignored a compound synthesized in the very beginning: the hydrochloride form of the later chlordiazepoxide. For a few years the team had no success at all, till colleague Earl Reeder reminded Sternbach about the almost ignored compound already synthesized in 1955. Sternbach was skeptical but Reeder got his own way. Product trials started in May 1957 and the 'Eureka!' came a few days later from pharmacologist Lowell O. Randall. He confirmed that the compound showed unusually interesting tranquilizing properties! Structure determination was started and it appeared to be a compound with the generic name methaminodiazepoxide, later changed into chlordiazepoxide. In 1960 it was introduced under the trade mark Librium, and Sternbach was regarded as the prime discoverer of the benzodiazepines, although Earl Reeder was actually a co-discoverer. The break through for Librium had taken place and it was the beginning of the benzodiazepine success story.
A broad program was developed aimed at finding products even superior to Librium. Soon, several newly synthesized compounds showed similar activity spectra, but varied in potency. The 1-methyl-derivative of chlordiazepoxide appeared to be the most potent of the produced compounds and this product, introduced in 1963, was given the generic name diazepam; the active component of Valium. Valium appeared to be the crown on Sternbach's work. All superlatives are given to this drug, which became the most widely prescribed and famous drug in the world. Without any competition, it was the most safe and effective therapeutic drug for anxiety and related disorders. It became an essential medicine on the list of the

World Health Organization. Valium is regarded as, 'the drug that changed the world'. In the words of the Rolling Stones, Valium pills are 'mother's little helpers' and Valium is seen as a 'sunglass for the soul' [4]. After Valium, Sternbach's team patented more benzodiazepines, such as in 1965 the hypnotic nitrazepam (Mogadon), in 1968 the anxiolytic medazepam (Nobrium), in 1973 the anticonvulsant clonazepam (Rivotril), in 1975 the hypnotic flunitrazepam (Rohypnol), in 1978 the hypnotic flurazepam (Dalmadorm) and in 1982 the short acting hypnotic midazolam (Dormicum) (Figure 2). In each and every substance, anxiolytic, hypnotic, sedative, anticonvulsive and muscle relaxing properties are present, but the copmpounds vary in the degree to which these different elements are expressedin the preclinical and clinical profile. Many other pharmaceutical companies joined the race in the creation of compounds with a similar therapeutic profile and in the course of time almost 50 new 'me-too' benzodiazepines were created. Moreover, when the patent on Valium ended in 2002, many drug companies came up with their own version of Valium under the generic name diazepam. Untill two years before his death at an age of 97 (September 28 2005), Sternbach was involved in benzodiazepine research and continued to go to his office daily, even after his retirement in 1973.

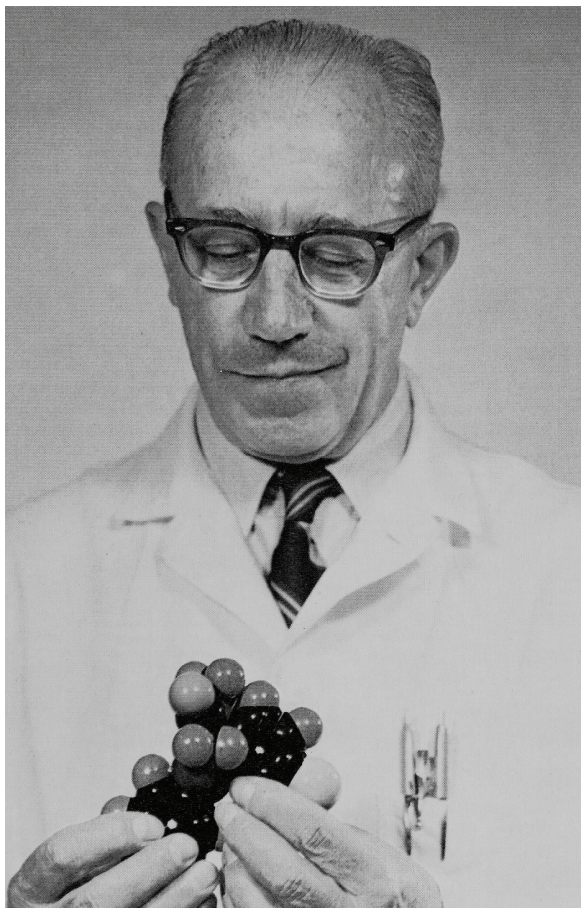

Figure 1. Leo Henryk Sternbach, around 1975, with the golden drug Valium in his hands [1].

EPILOGUE

The mechanism of action of the benzodiazepines became clear during the last few decades[4]. Benzodiazepines interact with the GABA receptor complex. GABA is the main inhibitory neurotransmitter of the brain and benzodiazepines facilitate its action by allosteric modulation. With this in mind it is understandable that benzodiazepines, as GABA agonists, help in situations where the action of the inhibitory neurotransmitter is too weak, like in anxiety, insomnia, epilepsy and high muscular tension. Benzodiazepines are effective and powerful medicines. Over the years their negative side-effects such as sedation, amnesia, drug dependence and negative effects on (e.g. car driving) performance have also become recognized. This has resulted in a search of pharmaceutical companies for more specific drugs, for example anxiolytics without sedative effects. But although some improvements have been realized, this goal has not yet been fully reached - all newly designed non-benzodiazepine medications are still not specific enough or completely spared from the benzodiazepine side effects.

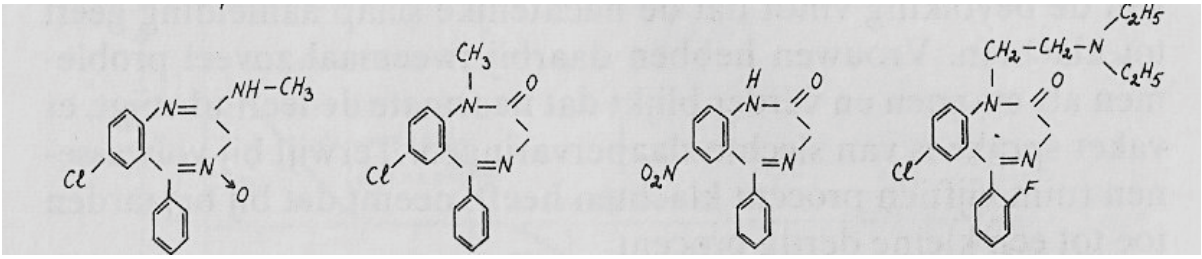

Figure 2. The structural formulas of four Roche benzodiazepines. Left: chlordiazepoxide (Librium), middle left diazepam (Valium), middle right nitrazepam (Mogadon) and right flurazepam (Dalmadorm). Note the similar structure of the compounds.

The benzodiazepines received much negative publicity when their common use was scrutinized. They are often regarded as a panacea for various disorders with a psychological background and are unfortunately often prescribed for chronic use to subjects without clear medical symptoms. This has led to a loss of drug efficacy and far worse, dependency. Misuse and abuse are at such a level that the Dutch authorities started a program to reduce benzodiazepine usage. To heighten the threshold for its use, it was decided that doctors may only prescribe these drugs for a short time and only in moderate doses and, even more recently, authorities withdrew benzodiazepine drugs from reimbursement by medical insurers. Despite this recent negative approach from the authorities, one should acknowledge that benzodiazepines cannot be missed in daily practice and that they still are a wonderful gift from the psychotropic legacy of Sternbach. When he escaped from Europe to the United States, Sternbach could not have imagined the golden success story of the benzodiazepines, which started in Kraków and finished in Nutley.

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SLEEP-WAKE
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Research papers

NEUROPEPTIDE S ELICITS ACTIVE WAKING WITH COMPENSATORY EEG SLOW WAVE INTENSITY IN RATS

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INTRODUCTION

Ample evidence exists in the animal research literature that indicates a key role of neuropeptides in the regulation of complex functions of the brain. In addition, recent neurobiological research holds promise for neuropeptide receptors as therapeutic strategies in the development of selective and alternative psychopharmacological treatment of anxiety and sleep disorders (1;2;3). Neuropeptide S (NPS) is a peptide expressed in various tissues with highest levels in the brain, tyroid, salivary and mammary glands (4). Within the brain, the highest level is found in a few discrete nuclei of the brainstem pontine area, located between the noradrenergic locus ceruleus (LC) and Barrington's nucleus (4). Interestingly, the NPS receptor mRNA is expressed in the cortex, hypothalamic and amygdala structures: brain centers that regulate vigilance states and anxiety (5). Consistent with this pattern of expression, it has been shown that intracerebroventricular (icv) administration of NPS produces a long-lasting arousal and modulates anxiety related behavior, facilitates spatial memory, increases wakefulness and decreases sleep in rodents (4;6;7;8). Observations of mice carrying a targeted mutation in the NPS receptor gene revealed reduced arousal when compared to their wild type littermates, and thus provide additional evidence and confirmation of the stimulatory, anxiolytic, activity of NPS (9).
To further elucidate the role of NPS in EEG-defined waking, we examined in rats during the light period the effects of intracerebroventricular (icv) infusions of NPS (1 and 10 nmol) on six vigilance states and on EEG spectral components.

METHODS

Animals and surgery

Male Sprague Dawley rats (Charles River, France) weighing 250–300 g at the time of surgery were used in the polygraphic recording experiments. Rats were provided with a microchip for identification purposes, housed in individually ventilated cages, located in a sound-attenuated chamber. Animals were maintained under controlled environmental conditions throughout the study: 22 °C ± 2 °C ambient temperature, relative humidity 60%, standard 12:12 h light cycle regime (illumination intensity: ~ 100 lx) and had free access to standard laboratory food chow and tap water. All experimental protocols were carried out in strict accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) and were approved by the animal care and use committee of J& J PRD and local ethical committee.
Under isoflurane anaesthesia, adult rats (n=18) were chronically implanted with electrodes for recording the frontal and parietal EEG (AP + 2 mm, L - 2 mm, and AP - 6 mm, L +3 mm from Bregma, according to the atlas of Paxinos & Watson 1998), EOG (peri-ocular muscles) and EMG (nuchal muscles). A stainless steel guide cannula was placed into the left lateral

ventricle for the intracerebroventricular (icv) injections. The animals were housed in individually ventilated cages (IVC) maintained on a 12:12 light-dark cycle (lights on 12:00 am) under fully controlled environment.
After recovery, two experiments including a total of 18 operated animals which were randomly assigned to 2 treatment conditions were performed. Each experiment consisted in 2 recording sessions: the first recording session lasted 20 hours after the administration of saline, while the second recording session was performed in the same animals for the same duration but following administration of saline (n=9) or NPS (n=9 per dose). NPS dose in the first experiment was 1 nmol whereas in the second experiment a dose of 10 nmol was administered. In both experiment, the control group received while the second group of animals received different doses of NPS (n=9 for each dose).A wash-out period of at least 2 weeks was allowed to elapse between experiments to avoid possible transfer effects on vigilance states. Next, data from both experiments were grouped by condition.

Vigilance states and EEG power spectra determination
Six sleep stages were classified as being indicative of active wake (AW), passive wake (PW), light slow wave sleep (lSWS), deep slow wave sleep (dSWS), intermediate stage (IS) or rapid eye movement sleep (REMS). Different sleep-wake parameters were calculated and time spent in each vigilance state, latencies for light sleep, deep sleep and REMS are presented here.
The peptide-induced variations in EEG power spectra were determined in all active waking EEG artifact free epochs of 2-sec over the first hour after administration. The spectral changes were calculated as the ratio of mean spectral power obtained following the injection of the peptide (day 2) versus the mean spectral power obtained following administration of vehicle (day 1). This ratio was calculated over frequencies between 0.5 and 50 Hz for active waking and changes in slow wave EEG activity (0.5-4 Hz) during deep sleep were documented at hourly interval over 4 hours post-administration of NPS.

Statistical analysis
The time course of the different sleep variables following each treatment were expressed as the mean ± S.E.M averaged within each treatment group and presented as mean values over 30 minute periods. The time course profile of the effects of the peptide on EEG spectral profile was expressed as a percentage of the mean spectral profile for the active waking and for slow wave EEG activity in the baseline saline period. All data were submitted to Mann-Whitney test with Bonferroni correction and tests were conducted at the two-sided 5% risk levels using SAS software, version 9.1.3.

RESULTS
When administered centrally, at the acrophase of sleep, NPS at the different doses used in these experiments elicited consistent changes in sleep-wake organisation (Figure 1). During the entire recording period, NPS did not significantly affect total amounts of waking and sleep (data not shown). Closer analysis of time distribution among arousal and sleep stages indicates that acute icv administration of NPS at 1 and 10 nmol resulted in a significant increase in active waking (+88% and + 87%, $p<0.05$; respectively) during the first 2 hrs with no clear dose response action (See Figure 1). A related decrease in the amount of light sleep (-84% and -68%, $p<0.05$; respectively), deep sleep (-47% and -33%, $p<0.05$; respectively) and REMS (-71% and -70%, $p<0.05$; respectively) was observed during the first 2 hours following the administration.

Interestingly, the effects on light sleep lasted up to 8 hours after administration, with a gradual recovery (Figure 1). A concomitant increase in deep sleep was found, however the few significances obtained seems rather spurious and a clear deep sleep rebound, discrete in time following the initial activation could not be determined.
In terms of spectral patterns, an enhancement in slow wave intensity (absolute power 0.5-4 Hz) during deep sleep was observed as of the second hour after administration and the changes became more prominent with time particularly with the lowest dose of NPS (Figure 2). Thus, NPS induced changes after an initial wake-promoting effect in sleep intensity rather than sleep duration.

In addition, the wake promoting effects of NPS are consistent with marked increases in different sleep onset latencies (Figure 3). In contrast, examination of total number of transitions from sleep states towards waking did not provide evidence of sleep fragmentation (data not shown).

The quantitative electroencephalographic profile of EEG waking activity during the first hour after administration of NPS resulted at both 1 and 10 nmol doses in decreased synchrony in EEG oscillations in the frequency range of 5-7 Hz while the peptide enhanced synchrony in EEG oscillations in the frequency range of 8-10 Hz (Figure 4).

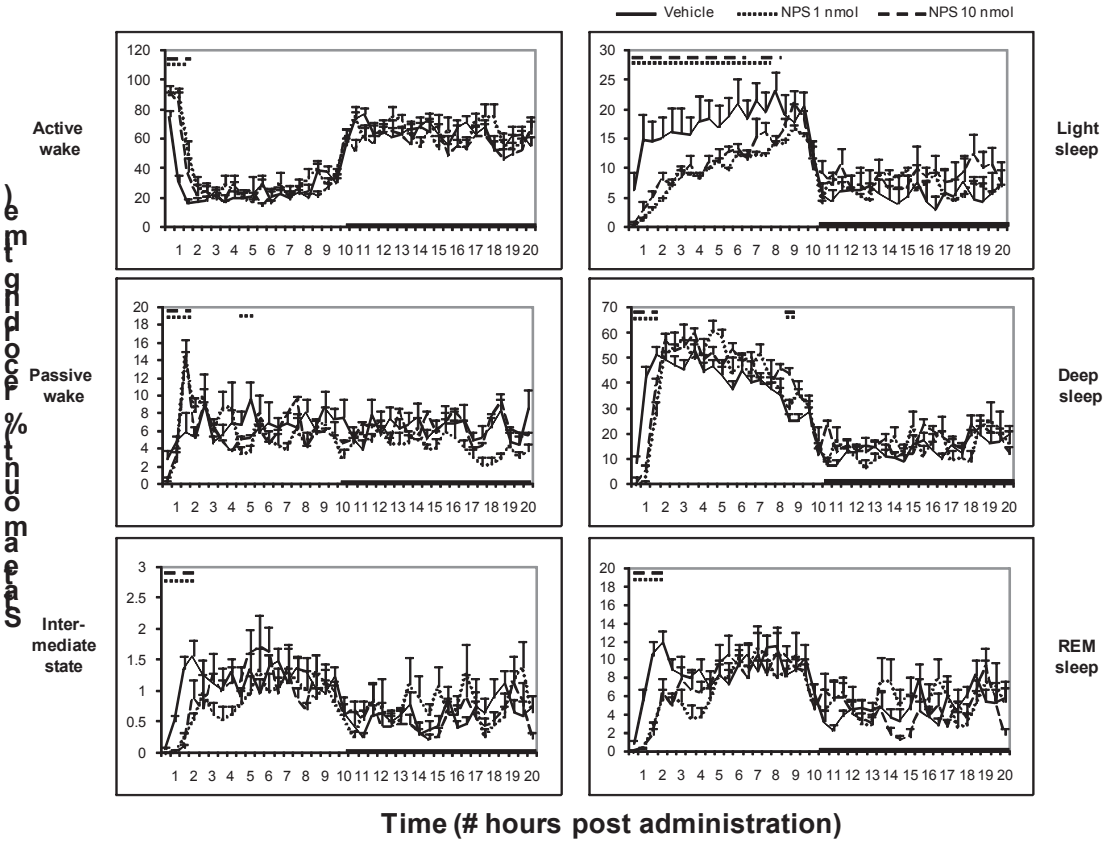

Fig. 1 *Percentage distribution of AW, PW, lSWS, dSWS, IS and REM sleep for each half hour period of the 20 h recording session following icv administration of NPS (1 and 10 nmol, n=9, each dose) or saline (pooled to n=18 for graphical simplification) . Open and dark areas in the abscissa axis denote light and dark phase, respectively. Bars at the top indicate intervals in which values differed between saline and peptide-dose (p < 0.05). Values are presented as means ± S.E.M. for each condition.*

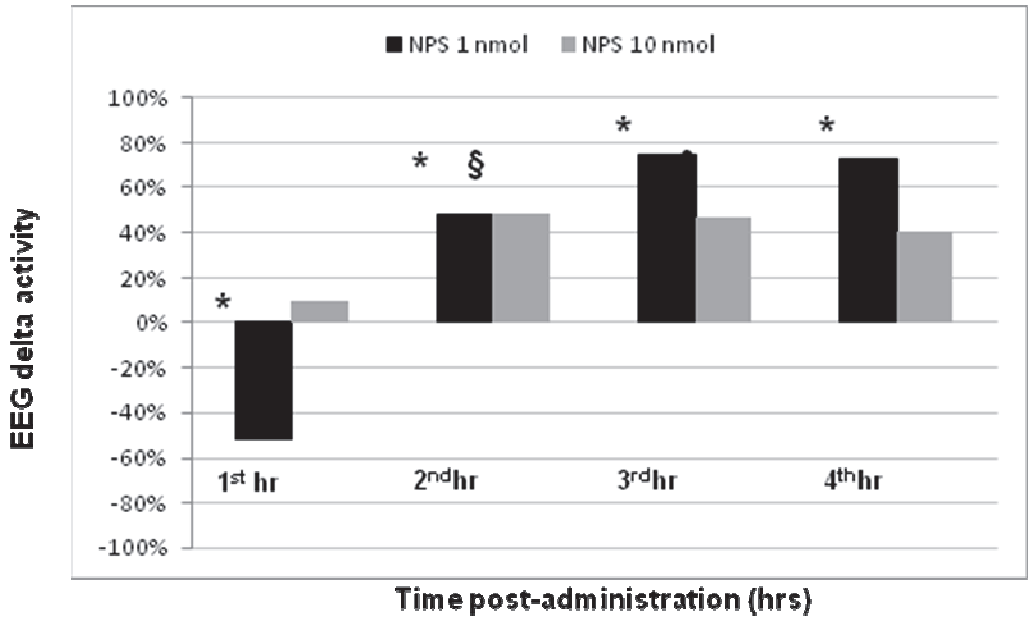

Fig. 2 *Changes in EEG slow wave activity (absolute power 0.5-4 Hz) during deep sleep in 1-hr time block after the administration of NPS. The ordinate gives the power in percentage of the baseline saline values (within-animal comparison) over the first four hours of the recording period. Each symbol indicates a statistically significant difference ($p < 0.05$) between saline and peptide-dose (* NPS1 nmol; § NPS 10 nmol).*

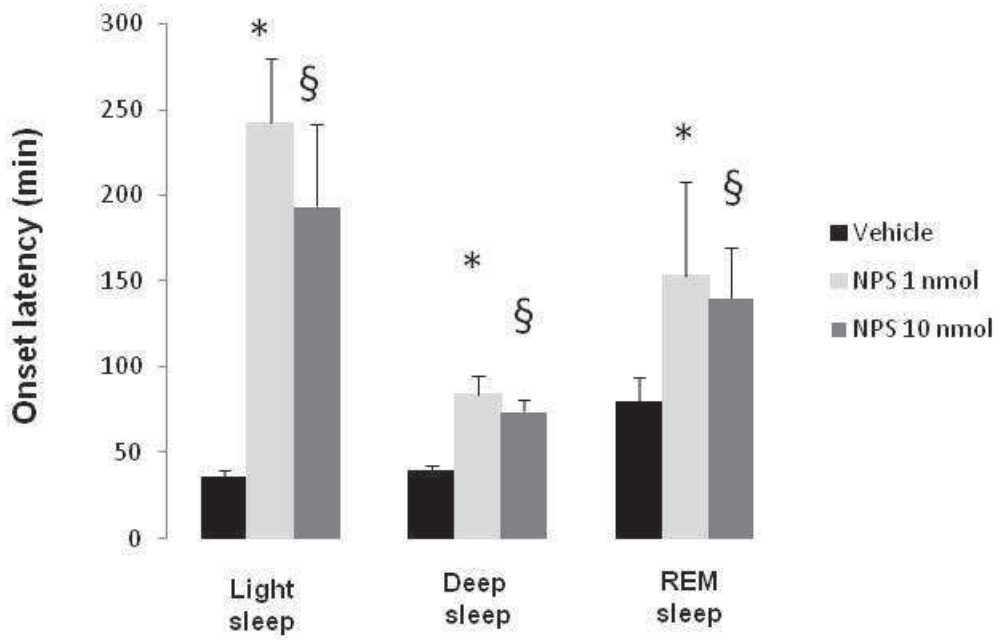

Fig. 3 *Effect of NPS on onset latency of sleep stages. Values are presented as means ± S.E.M. for each condition. Each symbol indicates a statistically significant difference ($p < 0.05$) between saline and peptide-dose (* NPS1 nmol; § NPS 10 nmol).*

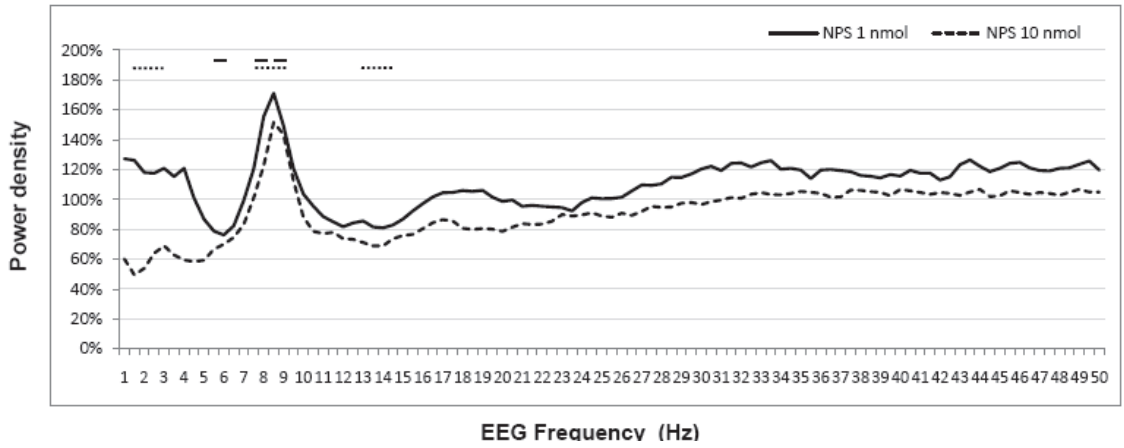

Fig.4 *Mean changes in spectral EEG power density (0.5-50 Hz) in AW after acute i.c.v. administration of NPS (1 and 10 nmol) expressed as percentage change over saline (within-animal comparison) over the first hour after administration. Lines at the top indicate intervals in which the peptide-dose effect differed ($p < 0.05$).*

DISCUSSION

The present study demonstrates that NPS evokes a robust and consistent wake promoting response in rats. This stimulating effect was associated with an enhanced synchrony in EEG oscillations in the frequency range 8-10 Hz during the first hour of active waking. In addition, effects on light sleep could last up to 8 hours after administration, while no clear intermediate or delayed compensatory deep sleep response was observed. Therefore, the present findings corroborate the arousal promoting profile described earlier for this endogenous ligand (4).

Similar findings of degree of stimulation i.e. onset and duration of arousal were described in mice and rats (4;10) showing that arousal and stimulating effects (11) of NPS is consistent across animal species and experimental conditions. Our sleep-wake findings parallel in part earlier findings in rats (4) in which the stimulating effects of NPS were reported during the first hour after administration of 0.1 and 1 nmol followed by a rebound during the second hour of the recording. In the present study, we have considered a high dose of 10 nmol, nevertheless, our observations over a 20 hours post-administration recording do not show a clear dose-response stimulating effect and does not support the finding on a marked overcompensation in the duration of sleep stages. However, the increased low-frequency intensity in the EEG during deep sleep, which likely represents a compensatory rebound response, was only seen consistently at a low dose of 1 nmol but not at 10 nmol. Therefore, sleep wake stage duration and EEG delta intensity seem to be coupled in a differential way to the doses tested of NPS.
Neuropeptides involved in the modulation of arousal and emotional states have been shown to be involved in the regulation of the hypothalamo-pituitary adrenal (HPA) axis. NPS increases arousal via its action in a number of distinct region within the brain including the HPA axis. Recent report indicate that NPS caused a marked stimulation of the HPA axis with

an increase in plasma ACTH and corticosterone, which occurred concomitantly with inhibition of food intake, effects suggested to be mediated through the release of corticotropin-releasing factor (CRF) (10). Indirect evidence suggested an implication of CRF in the regulation of physiological waking in the absence of stressors. Dynamics in plasma levels of cortisol/corticosterone and ACTH are temporally associated with waking and sleep i.e. are lowest before the major sleep time and highest at the beginning of the active period in humans and rats (for review see (12)), and central or systemic administration of CRF into rats and humans increases EEG-defined waking (13). However the unique pharmacological spectrum of NPS i.e. stimulant with anxiolytic-like activity in animals exposed to different stressful paradigms suggest that other pathways are indicated. A relatively high density of NPS receptor has been observed in the hypothalamic nuclei (5), a brain area where most of orexinergic neurons are located. Orexin peptide promotes profound wakefulness and suppresses sleep (14). A recent paper indicates activation of orexin-containing neurons following central infusion of NPS (15), which suggests that NPS neurons innervate the orexinergic neuronal system. Therefore it is plausible that effects of NPS may be mediated directly through activation of the orexin neurons to promote wakefulness and inhibit sleep.
NPS receptors are also expressed in hippocampus, implicated in learning and memory process. Recently, it has been demonstrated that NPS can facilitate spatial memory and inhibit MK801-induced spatial memory impairment in the morris water maze (MWM) in mice (8). Theta EEG rhythm is generally considered to be a reflection of underlying hippocampal versus other brain area synchronization. Theta oscillations accompany brain activity of explorative, mnemonic nature. The enhanced theta activity observed during peptide-induced wakefulness might thus reflect heightened alertness. Taken together our results suggest that the consolidated wake-promoting action of NPS may well correlate with putative cognition-enhancing effects.

The present study provides further evidence that NPS induces a short lasting increase in wakefulness and a probably secondary longer lasting shift from light sleep to deep slow wave sleep. A differential response to NPS treatment was associated with changes in EEG delta intensity during deep, but not with sleep stages duration. The peculiarities of wake promoting action of NPS with enhanced EEG synchrony in the frequency range of 8-10 Hz are considered putatively favorable features in wake-promoting agents.

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SLEEP DEPRIVATION BY GENTLE HANDLING IN RATS: IS IT STRESSFUL?

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INTRODUCTION

In today's society, sleep disturbances occur frequently among adults, adolescents as well as children, and are considered to be detrimental to good health. Controlled studies have, for example, shown that sleep loss acts on the stress systems, thereby sensitizing individuals to stress-related disorders (for review see[1]). It is thought that sleep loss may alter or chronically activate these systems, thereby releasing stress hormones which in turn have many different effects on a behavioral, emotional, cognitive, physiological, hormonal, brain functioning and metabolic level[1-3]. Via these stress processes, sleep loss can cause the well-described detrimental consequences on human health. Therefore, it is relevant to study the complex and bi-directional relationship between sleep loss and activity of the stress systems.
The present study examined the effect of short-term 4 h sleep deprivation induced by gentle handling in rats, on physiological (heart rate, body temperature and locomotor activity) and hormonal (corticosterone) stress parameters together with the recovery thereof. The stress of the procedures used for measuring these parameters or used to induce sleep deprivation can be a serious confounding factor in these types of studies. Therefore, this study aimed to take measurements in a way that would minimize possible confounding stress effects of the sampling and sleep deprivation procedure to be able to see the effects of sleep loss per se.

METHODS

Animals and surgery

Male Sprague-Dawley rats (Harlan, The Netherlands), weighing 310-330 g at the time of surgery were used. These rats were singly housed under 12 : 12 h light/dark cycle with a 30 min dim/rise period. Food and water were available ad *libitum*. The rats were implanted with a biopotential transmitter (DataSciences International) to record heart rate, body temperature and locomotor activity. Together with this, a catheter for blood sampling was implanted in the jugular vein. After surgery, the animals were allowed to recover for at least two weeks. During the subsequent experimental period, potentially disturbing stimuli were carefully avoided.

Set-up and measurements

The experimental set-up allowed assessment of different parameters simultaneously from the same freely moving rat under strictly controlled, undisturbed, home-cage conditions with minimized confounding stress factors due to sampling procedures. It consisted of a telemetry

set-up (DataSciences Int.) to measure continuously and automatically physiological parameters using the implanted transmitter. Concomitantly, blood was sampled automatically and repeatedly through the jugular vein catheter using an automated blood sampling machine (AccuSampler® Micro; DiLab®). The blood samples were stored on -80°C until radioimmunoassay analyses for corticosterone (125I RIA Kit, MP Biomedicals, LLC). Sampling for all measurements started 30 min before onset of the sleep deprivation and lasted until 3 h 40 min after termination of the sleep deprivation.

Sleep deprivation

The animals were subjected to either control conditions (n=7) or sleep deprivation (n=7) using a cross-over Williams design. Sleep deprivation was induced at lights-on (resting phase) starting with a 30-min rise period, by using the gentle handling method according to those used by the group of Grassi-Zucconi[4]. Whenever the animal showed signs of sleep (attempting to engage a sleep posture or drowsy look) they were stroked on their back or moved. Only when really necessary the animals were held in hand briefly. This method is known to effectively induce wakefulness for at least 80% of the sleep deprivation period[4]. The sleep deprivation was terminated following the t = 240 min blood sample. Therefore, the sleep deprivation lasted around 4 h and 5 min, which is described as 4 h. Under control conditions, animals were left undisturbed in the same experimental room.

Statistical analyses

For the telemetric recordings and corticosterone measurements, statistical significance was evaluated using a repeated measures ANOVA with treatment and time as factor variables. In addition, period effects as well as possible carry-over effects were accounted for. P-values were adjusted for multiple comparisons using the false discovery rate. All tests were performed at a significance level of 0.05.

RESULTS

Under control conditions, locomotor activity declined rapidly at the start of the light period, reaching stable levels around a mean level of 0.76 ± 0.06 counts, which indicates overall inactivity of these animals. Heart rate levels decreased gradually and reached stable values around a mean level of 360.4 ± 1.0 bpm. Body temperature decreased slowly after lights-on until reaching stable values around the mean level of 37.6 ± 0.0 °C. Plasma corticosterone levels increased slightly and then were found to stay around a mean level of 29.7 ± 2.7 ng/ml. Sleep deprivation by gentle handling induced a significant increase in locomotor activity, heart rate and body temperature compared to the control condition, but not compared to baseline (i.e., the last 30 min before lights-on) (Figure 1A-D). This indicates that levels comparable as to before lights-on were maintained during sleep deprivation. At termination of sleep deprivation, activity and heart rat returned to stable control values within 5 min. Body temperature, however, decreased more gradually reaching stable control values only 30 min after the end of sleep deprivation. Corticosterone levels were elevated as compared to the control animals during the sleep deprivation period, but this increase only reached significance during the last 5 min of the sleep deprivation. Upon termination of the procedure corticosterone levels stayed higher for another 10 min until reaching again control levels and even going below control levels one hour later for 30 min.

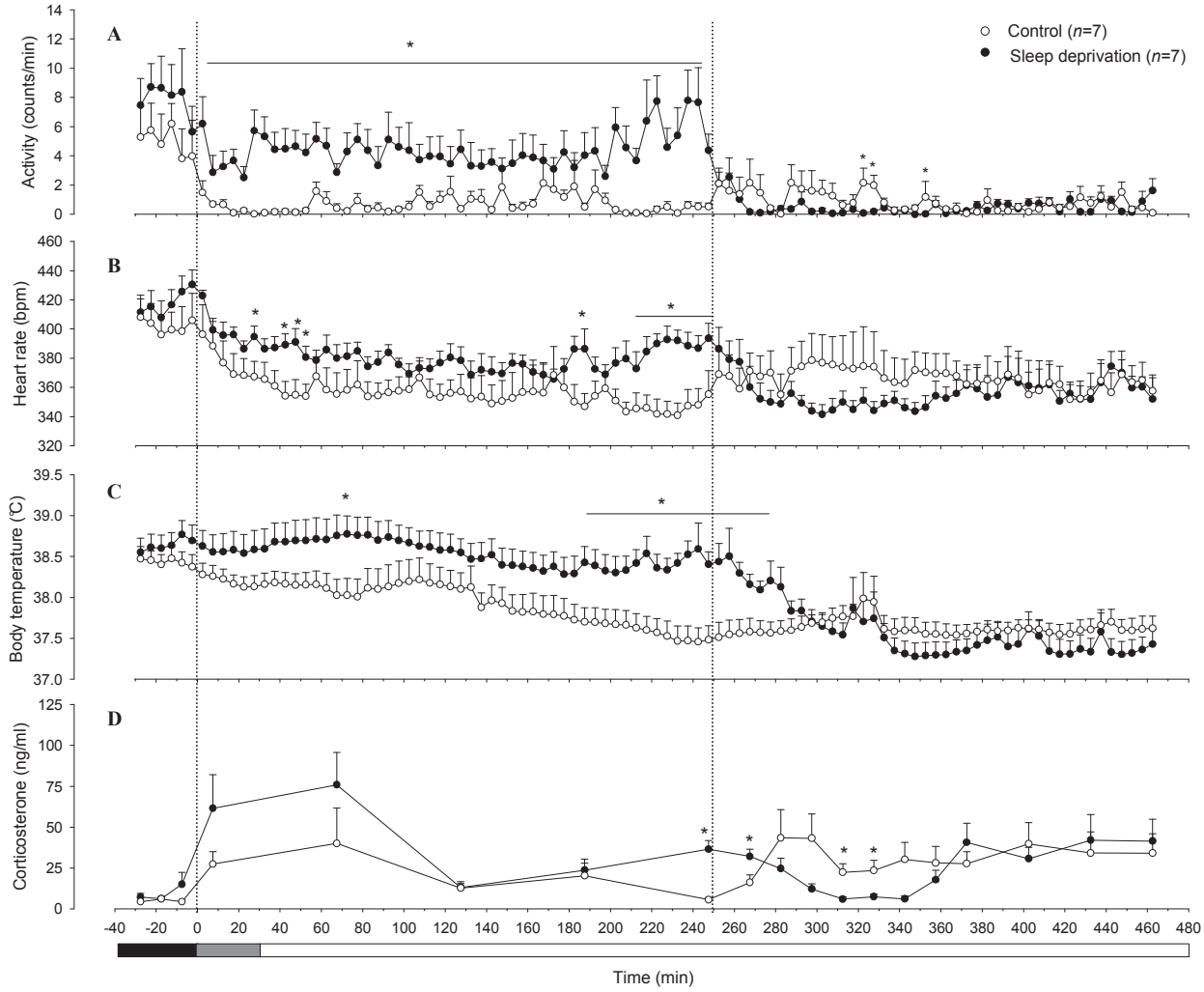

Figure 1. Time course of the changes in locomotor activity (**A**), heart rate (**B**), body temperature (**C**) and plasma corticosterone (**D**), for the animals exposed to 4 h sleep deprivation (*n*=7) and for the control condition (*n*=7). Data are expressed as 5-min averages + SEM for the physiological measurements and for every 10-60 min + SEM for the corticosterone measurements. The responses are shown for the last 30 min of the dark period, the 4 h (+ 5 min) sleep deprivation period (indicated by area between the lines) starting at lights-on with a 30-min rise period, and the 3 h 40 min post-sleep deprivation period. The bars underneath the graph indicate the dark (black), the 30-min rise (gray) and light (white) period. *type effect: sleep deprived versus control, $p<0.05$. All showed significant time*treatment effect.

DISCUSSION

The 4 h sleep deprivation by gentle handling, at the beginning of the light period, induced locomotor activity, heart rate, body temperature and plasma corticosterone to stay above those of the undisturbed sleeping controls. The increases were mild and reached levels comparable to those at the end of the dark period. Upon termination of the sleep deprivation procedure, all variables eventually returned to control values but showed different time courses. Since all the measured physiological and hormonal stress parameters reached stable control values within 30 min upon termination of the sleep deprivation and because the

deprivation values did not exceed levels seen during the active (dark) period, the physiological and hormonal effects of short sleep deprivation by gentle handling can best be classified as mild.

This observed mild activation of the stress systems was most likely unaffected by the applied sampling procedures. This is confirmed by the normal and stable measured physiological values together with the low plasma corticosterone values under control conditions, indicating that the sampling methods used have indeed reduced possible confounding stress factors. Therefore, the observed mild physiological and hormonal effects are likely to be a true effect of sleep deprivation and sleep loss, but on the other hand, they may also be an effect of the method used independent of sleep loss. It is considered to be difficult to fully separate these two (as discussed by Meerlo et al.[1]). To limit the possible stress confounding effects caused by the sleep deprivation procedure in this study, the gentle handling method was used. This method is considered to significantly reduce sleep time without being a major stressor. In agreement with this assumption are the mild increases in the measured physiological and plasma corticosterone levels during the sleep deprivation period in this study. In fact, the measured levels stayed below those seen during mild stress[5-7]. Stress generally increases our measured parameters[2]. More specifically, activation of the two major neuroendocrine stress systems, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathico-adrenomedullary system, result in the release of the glucocorticoid corticosterone and the catecholamines adrenalin and noradrenalin into the bloodstream[2]. These in turn increase the blood flow to the muscles by increasing the output of the heart and through vasodilatation, thereby increasing blood pressure, heart rate and eventually also body temperature. The activity of the animals may also affect the measured physiological and hormonal levels[2]. Even though our measured activity levels show that spontaneous activity was induced during the whole sleep deprivation period, these levels were still low compared to true exercise levels[7]. Thus, according to our measured parameters, the gentle handling sleep deprivation method managed to minimize possible confounding stress effects, by affecting the stress systems only mildly and by inducing only low levels of spontaneous activity.

In conclusion, stress does not appear to be a confounding factor in our method of gentle handling sleep deprivation, and 4 h sleep loss per se affects the physiological and hormonal stress parameters only mildly.

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MELATONIN DECREASES DAYTIME CHALLENGING BEHAVIOR IN PERSONS WITH INTELLECTUAL DISABILITY AND CHRONIC INSOMNIA

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INTRODUCTION

Persons with intellectual disability (ID) and sleep problems exhibit more daytime challenging behaviors, irritability and lethargy than persons with ID without sleep problems[1]. The relationship between challenging behavior and sleep problems is complex. Daytime challenging behavior may both be a cause and a result of sleep problems, or it be caused by a third independent variable[1].
Melatonin improves sleep in ID patients[2].There are several anecdotal reports suggesting that melatonin also decreases daytime challenging behavior[3], but randomized controlled trials (RCT) have not been conducted yet.
The aim of this study, therefore, was to investigate the effects of melatonin treatment on daytime challenging behavior and to explore associations between daytime challenging behavior, chronic insomnia and melatonin circadian rhythm in persons with ID. We hypothesized that (a) melatonin treatment is more effective in reducing daytime challenging behavior in ID persons and chronic insomnia than placebo, and (b) there is a strong correlation between the presence and severity of challenging behavior and the severity of sleep problems at baseline, as well as a strong correlation between changes in challenging behavior and changes in sleep parameters between baseline and the end of the treatment period.

METHODS

Patient data of two previously published RTC's on the efficacy of melatonin in persons with ID and chronic insomnia, using identical methodology and performed within the same time window (2000 – 2004), were combined[4,5]. In sum, after a baseline week participants received either 5 mg melatonin (≥ 6 years) at 7pm, or 2.5 mg melatonin (< 6 years) at 6pm, or placebo during 4 weeks. During the baseline week and consecutive treatment weeks caregivers completed a sleep log to assess sleep on a daily basis. Dim Light Melatonin Onset (DLMO) was measured in saliva at the last night of the baseline week and at the last night of the last treatment week.

Challenging behavior

To measure daytime challenging behaviors, parents and other caregivers completed the *Storend Gedragsschaal voor Zwakzinnigen* (SGZ; Maladaptive Behavior Scale for the Mentally Retarded)[6] in the baseline week and at the end of the fourth (i.e. last) treatment week. The SGZ consists of 32 items, and three subscales: (a) Aggressive maladaptive behavior (SGZ-A, maximum score of 72), (b) Verbal aggressive behavior (SGZ-V, maximum score of 28) and (c) Mixed maladaptive behavior (SGZ-M, maximum score of 160). The SGZ-A contains 8 items on scratching, pinching, biting, pushing, hitting, kicking, spitting or pulling hair of parents, caregivers, siblings and/or other persons. The 5 items of the SGZ-V are on abusive language and nagging towards caregivers and/or other persons. The SGZ-M contains 19 items on self-injurious behavior, stealing food, pica, tearing clothes, throwing or destroying objects, hyperactivity, screaming, nagging, noncompliance, public masturbating, faeces smearing, coming out of bed at night with waking others and stereotyped behaviors. Each item is rated on a 4-point scale, ranging from 0 (behavior did not occur) to 4 (behavior occurs at least 5 days per week). Scores of most items are multiplied with 2 or 3, depending on the degree that the specific item is disturbing to others. Total scores on the three subscales are based upon summing the scores of the subscale items. Scores from the three subscales may be summed to attain a total score (i.e. SGZ-T), which reflects the overall severity level of challenging behavior (maximum score 260). The SGZ is validated for ID persons, aged 3 years and over, and IQ 70 or less.

Outcome measures

Primary outcome measures were SGZ scores for the measurement of challenging behaviors, and time lights went out, sleep latency, total sleep time and number and duration of night wakes that provided a measure of sleep quality. Secondary outcome measure was DLMO to assess circadian rhythmicity.[13]

Statistical analyses

Independent samples *t*-tests were used to test whether there were differences in outcome measures between the melatonin and placebo group at baseline. T-tests for independent samples were also conducted to test differences in change between melatonin versus placebo treatment on the SGZ scores, DLMO and sleep parameters. In cases where equal variances could not be assumed (tested with Levene's test) results of t-tests with adjusted degrees of freedom are reported. GLM analysis corroborated the findings of the t-tests. Relationships between SGZ scores, DLMO and sleep variables were explored using Pearson's correlation coefficient. Statistical significance was accepted at $p < .05$.

RESULTS

Sixty-six children and adults with ID and chronic insomnia were randomly assigned to melatonin or placebo conditions. During the baseline week parents of one child withdrew informed consent because their child refused to cooperate with saliva sampling. Nine individuals in the placebo group and seven in the melatonin group were excluded because parents or other caregivers did not complete the sleep logs or SGZ forms. The melatonin group consisted of 16 males and 11 females, mean age 18.8 years, SD = 18.0. The level of ID was profound (n = 11), severe (n = 9), moderate (n = 3), and mild (n = 4). The placebo group consisted of 9 males and 13 females, mean age 17.5 years, SD = 16.5. The level of ID was profound (n = 9), severe (n = 4), moderate (n = 3) and mild (n = 6).

Table I Difference in change in DLMO, SGZ scales and sleep parameters

	Melatonin						Placebo							
	Baseline			Treatment		Change	Baseline			Treatment		Change		
	n	Mean	SD	Mean	SD	Mean	n	Mean	SD	Mean	SD	Mean	Diff. In change	*p*- value
SGZ-A	27	13.33	16.39	9.52	11.34	-3.81	22	9.00	15.91	8.36	15.18	-0.64	-3.17	.076
SGZ-V	27	2.89	5.58	2.15	4.58	-0.74	22	2.41	5.61	1.95	5.19	-0.45	-0.29	.617
SGZ-M	27	40.59	27.57	30.81	22.28	-9.85	22	40.05	21.66	38.23	21.78	-1.95	-7.90	**.003****
SGZ-T	27	56.81	40.26	42.48	29.93	-14.41	22	51.45	34.38	48.41	34.60	-3.05	-11.36	**.005****
DLMO[1]	26	21:32	1:35	18:54	1:12	-2:38	18	21:32	1:58	20:51	1:36	-0:41	-1:57	**.001****
Time light out[1]	27	20:43	1:04	20:33	1:45	-0:10	21	21:04	1:18	21:01	1:15	-0:03	0:07	.697
Sleep latency[2]	27	65	42	24	20	-40	22	53	36	48	39	-6	-34	**<.001****
Number of wakes/night	25	1.75	1.25	1.23	1.01	-0.52	20	1.47	0.82	1.61	1.09	0.14	-0.66	**.002****
Duration of wakes[2]	24	34	37	24	37	-10	20	17	21	16	24	-1	-9	**.034***
Total sleep time[1]	24	9:01	1:26	9:54	1:03	0:53	19	9:09	1:10	9:28	1:04	0:19	0:34	**.043***

1=hours:minutes; 2=minutes
* $P < .05$; ** $P < .01$

Compared to placebo, melatonin treatment significantly reduced mean SGZ-T and SGZ-M scores, but scores on the SGZ-V subscale remained unchanged. Mean SGZ-A score did not decrease significantly ($p = .076$) (Table I). SGZ items that showed a significant reduction in mean scores ($p < .05$) were hitting/kicking of parents/caregivers ($M_{melatonin} = -0.33$, $SD_{melatonin} = 0.68$; $M_{placebo} = 0.00$, $SD_{placebo} = 0.00$; $t\ (26) = 2.55$, $p = .017$), hitting/kicking of others ($M_{melatonin} = -0.26$, $SD_{melatonin} = 0.53$; $M_{placebo} = 0.05$, $SD_{placebo} = 0.21$; $t\ (35.75) = 2.75$, $p = .009$) and destroying objects ($M_{melatonin} = -0.63$, $SD_{melatonin} = 1.12$; $M_{placebo} = -0.09$, $SD_{placebo} = 0.29$; $t\ (30.36) = 2.41$, $p = .022$).

The influence of melatonin on sleep parameters is summarized in table 1. At baseline there were no significant correlations between SGZ-T scores, DLMO and sleep parameters, except for a negative correlation between SGZ-T scores and time lights went out at bedtime ($r = -.34$, $p = .018$), and a negative correlation between the SGZ-T scores and age ($r = -.23$, $p = .037$). After 4 weeks of melatonin treatment, change in SGZ-T scores did not correlate significantly with change in DLMO, nor with change in any of the sleep parameters (table II).

DISCUSSION

Melatonin in ID patients with chronic insomnia was more effective in reducing challenging behavior and sleep problems than placebo. This is the first randomized study to support clinical experience[5] that melatonin treatment not only improves sleep, but also reduces daytime challenging behavior in ID persons with chronic insomnia. These findings are in agreement with another study showing that melatonin improves daytime functioning[3].
Improvement of behavior after effective treatment of disordered sleep in ID persons is generally assumed to be caused by the improvement of sleep. However, in the present study presence and severity of challenging behavior did not correlate with severity of sleep problems at baseline, nor did we find a significant correlation between these variables at the end of the treatment period. Patients with severe sleep problems did not show more challenging behaviors than patients with milder sleep problems. Furthermore patients that showed a larger reduction of sleep problems after 4 weeks of treatment, did not also show a larger reduction of problem behaviors.

Although the correlation coefficients between change in SGZ-T scores with change in number of nights with wakes per week and with change in DLMO were statistically not significant, the relative high correlations (i.e > .30) between both parameters/variables may be of interest. Therefore it is plausible that the improvement of behavior is not caused by one

single factor, such as the improvement of sleep, but also by the correction of a disturbed melatonin circadian rhythm, as a second variable. The relation between change in SGZ-T scores and change in DLMO respectively number of nights with wakes per week points in this direction.
An improvement (i.e. decrease) in challenging behaviour might be attributed to the chronobiotic action of melatonin[7]. If so, challenging behavior in our ID patients not only were caused by poor sleep maintenance (in terms of?), but also were related to a misalignment or other disturbance of the circadian melatonin rhythm.

Another possibility is that melatonin has two separate working mechanisms: one which regulates sleep-wake rhythm and a second which improves behavioral functioning. Recently Jan et al. suggested that the thalamus plays an important role in the pathophysiology of circadian rhythm sleep disorders and the principles of sleep hygiene[8]. Maybe melatonin improves behavior indirectly by influencing thalamic functions.
Results of our study suggest that melatonin treatment can be effective in ID patients with chronic insomnia and challenging behaviors. Further studies are needed to confirm these findings.

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SLEEP QUALITY AND BODY MOTILITY OF HEALTHY SUBJECTS SLEEPING ON TWO TYPES OF MATTRESSES

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INTRODUCTION

It is well known that a sleeping person does not lie completely still during the long nightly sleep period. An estimation of Kleitman[1], based on several types of bed displacement measures, is that a sleeper shows about 30 seconds of motility per hour, with a total of 20 to 60 shifts in body position per night. Motility in sleep is further studied by video recordings and by interval photography [2, 3] as well as by actigraphy [4, 5]. Movements in sleep seem necessary to prevent stiffness of joints and to maintain an adequate blood supply. Less activity during sleep often indicates the use of psychoactive drugs, such as alcohol and sleeping pills [4], while more nightly activity is associated with impaired sleep [3]. Tossing and turning are features of disturbed sleep and insomnia.
Since detailed data of nightly activity are scarce, the present study focused on sleep motility in relation to the quality of sleep in healthy good sleepers. This was done to establish the movement features during the sleep period. In order to rule out eventual effects of the type of mattress, sleepers slept on two modern, comfortable mattress types, a Tempur-Pedic mattress and an Auping Pocket spring mattress. The Tempur mattress was chosen because the visco-elastic temperature sensitive material gives a subject a deep lying position, enclosing the body, which might not favour nightly movements. On the other hand the Auping Pocket spring mattress has a more firm surface, enabling a subject to lay on top of the mattress, which might allow sleep movements. The primary question was how many body position shifts and gross movements occur during a night of good sleep, and the secondary question was whether the type of mattress might influence the sleep quality and motility.

METHODS

Eighteen Dutch healthy 'middle-class' persons, who where self pronounced 'good sleepers', 8 men and 10 women, aged between 23 and 46 years and body weights between 65 and 90 kg, participated and signed an informed consent. Alcohol and drugs were prohibited on days preceding the experimental nights and coffee was no longer allowed after 18.00. Subjects slept 3 successive nights in the two- rooms sleep facility of the University of Amsterdam. Every night two subjects were measured. Room temperature was 19-21 ° C, with a humidity of 60-65%. After a habituation night, the first and second experimental nights were studied. Subject A arrived at 22.30 at the sleep facility, electrodes were fixed, an actigraph (Actiwatch) was attached and a body position sensor (Pro-Tech, Embla) was put on a belt around the chest. At 23.30 subject A went to bed, connected to the equipment, and room lights were switched off. Subject B arrived at 23.00, got the same procedure and lights were switched off at 24.00. All measurements, including video-recordings, started when the lights were switched off. At 07.30 subject A was awakened and filled in a sleep quality scale [6],

while subject B was awakened at 08.00 with the same procedure. During the habituation night the subjects slept on a latex mattress, having features in between the Tempur and the Auping mattresses. During the first experimental night half of the subjects slept on the first mattress and the other half on the second one, while this order was reversed on the second experimental night. Subjects were not aware of the details of the study and were also not aware of changes of mattresses. Paired sample t-tests compared the differences between the two mattress types.
Nightly activity of the subjects was measured with an actigraph. To determine turnings and shifts of the subjects body position in bed was determined with a body position sensor, distinguishing supine, prone, left and right body positions. All data were analysed over a period of 6 hours, between 00.30 and 06.30. An infrared video camera recorded continuously the subjects (sleep)- behaviour. The videotape was scored off-line for the same time segment by an analyst, who was blind-to-the-experiment. In this analysis focus was given to the scoring of gross movements and to complete turns (from supine to prone position, or reversed, and from one side to another) and half turns. Sleep was established by polysomnographic measures. The EEG was registered on two central locations (C3-A1 and C4-A1), simultaneously with the chin EMG, and the EOG. In this way objective sleep data were obtained. Subjective impression of sleep quality was obtained by a sleep quality scale (Visser et al. [6]).

RESULTS AND DISCUSSION

Table 1 shows the polysomnographic sleep data of 11 subjects (7 subjects did not get polysomnographic recordings), who slept one night on a Tempur and one night on an Auping mattress. No significant differences were found in these variables. Therefore the pooled data are also shown.

	TEMPUR	AUPING	POOLED
Sleep latency	12.4 ± 5.6	19.8 ± 18.5	16.1 ± 13.8
Sleep efficiency	94.5 ± 3.1	93.1 ± 7.3	93.8 ± 5.5
Total sleep time	429 ± 19	424 ± 48	427 ± 32
% Stage 1	7.2 ± 3.6	7.3 ± 3.8	7.3 ± 3.7
% Stage 2	50.2 ± 4.1	51.7 ± 4.5	51.0 ± 4.3
% Stage 3	10.4 ± 3.6	10.9 ± 4.3	10.7 ± 3.5
% Stage 4	6.6 ± 4.3	5.3 ± 3.7	5.7 ± 3.8
% REM sleep	18.8 ± 4.7	18.3 ± 6.5	18.6 ± 5.6
% non-REM sleep	79.9 ± 4.7	81.0 ± 7.4	80.5 ± 6.1
Number of awakenings	9.1 ± 4.8	9.7 ± 6.0	9.4 ± 5.3

Table 1. Polysomnographic sleep data of 11 subjects during the entire night (mean and SD)

In Table 2 the actigraphic data, the body position sensor data, as well as the video data are presented. No difference was found in total activity in the registration period from 00.30 and 06.30, in subjects sleeping on the two types of mattresses. Also the shifts in body position were established over the night of 6 hours. Whole and half turns were counted, both based on

the body position sensor data and also based on the video recordings. The result was approximately the same for both types of recordings. Besides the number of the whole and half turns, the gross movements of body, arms and legs were also observed from the video recordings. In Table 2 the total number of gross body movements are presented under the heading 'nightly activity'.

	TEMPUR	AUPING	POOLED
Total activity (n=18)	2947 ± 1807	3100 ± 1512	3023 ± 1644
Shifts body position (n=11)	13.6 ± 6.7	15.6 ± 6.6	14.3 ± 6.4
Shifts with video (n=15)	13.2 ± 4.6	12.9 ± 5.2	13.1 ± 4.8
Nightly activity (n=15)	29.7 ± 12.1	28.2 ± 8.5	29.1 ± 10.1

Table 2. Data of the actigraph (total activity), and of the shifts in body position established with the position sensor and the video recordings. Also the 'nightly activity' is indicated. All data are scored between 00.30 and 6.30

Directly after waking up 18 subjects filled in a 'sleep quality scale' [6]. Subjects estimated their sleep latency, their number of nightly awakenings (WASO), their wake time, as well as their sleep quality.. For their estimation of sleep quality subjects had to answer 'yes' or 'no' on 14 questions. Maximal score was 14 and the higher the score the better the quality of sleep. Estimated values were around 12, indicating a high quality of sleep. Again no significant differences were found and for this reason also the pooled data are shown.

	TEMPUR	AUPING	POOLED
Sleep latency (minutes)	14 ± 7.3	18 ± 12.6	16 ± 10.4
WASO numbers	2.3 ± 1.7	2.7 ± 2.2	2.5 ± 2.0
Wake time (minutes)	13 ± 22.1	12 ± 19.6	12.5 ± 20.6
Sleep quality (scale 0-14)	12.2 ± 1.5	11.6 ± 2.0	11.9 ± 1.8

Table 3. Subjective experience of sleep (mean and SD)

The quality of sleep of all subjects was high on both the Auping and the Tempur mattress. This was indicated by the objective sleep data, which were all in the range of the normative data in the authoritative publication of Kryger et al.[7], and was also confirmed by the subjective sleep estimates. The unfamiliar sleep environment and the recording devices seem not to have influenced sleep, except that sleep latency tended to be slightly longer than normal. Sleep efficiency was approximately 95%, suggesting that sleep of subjects was complete normal. It turned out that the type of mattress did not have an influence on the quality and quantity of sleep activity and has also no effect on the quality of sleep. No single difference was found, between the mattress which was claimed to reduce sleep movements and the mattress which allowed sleep movements. This is in agreement with the main results in the literature: a quality mattress with a quality surface does not influence sleep [8, 9]. An

exception is when the mattress is too hard and becomes uncomfortable. This will cause the nightly motility to increase with a lower subjective quality of sleep [10]. Unsuitable mattresses are regarded as a source of impaired sleep, but modern, comfortable mattresses do not cause sleep problems in healthy people. Bader and Engdal [11] state that personal preferences are decisive in choosing a soft or a firm mattress. It can, however, not be excluded that for people with back problems or with posture complaints it is better to use a specific type of mattress which makes it easier to move.
Since no single difference between mattress types was found in motility parameters, data were pooled. Nightly movements and turns were identified by the body position sensor and by the video recordings. Complete turns, from one side to another, or from back to belly, are on the average 2 per hour, with a total of 16 for a night of 8 hours. When incomplete turns, from one side to the back or belly or vice versa, and gross position shifts, such as from a stretched position to a bended position, or reversed, are added to the complete turns, it can be stated that approximately 30 position shifts occur per night.

CONCLUSIONS

All findings are in line with the literature findings, indicating that good sleep is associated with a moderate number of shifts and turnings in sleep position. All in all approximately 30 of such gross movements occur in a night of good sleep. A comfortable quality mattress has no direct effect on sleep quality and the number of these movements.

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DIURNAL RHYTHMS IN EPILEPSY ORIGINATING FROM THE TEMPORAL AND EXTRA-TEMPORAL LOBES

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INTRODUCTION

Numerous studies have focused on sleep and epilepsy and describe clear influences of epilepsy and seizures on sleep and vice versa [1]. Knowledge about the interaction of circadian rhythm and epilepsy is, however, relatively scarce [2]. It has been observed, that seizure occurrence seems to have 24h rhythmicity in humans and animals [3,4]. An important finding in a rodent model of limbic epilepsy was that a true endogenously mediated circadian rhythm in seizure occurrence was shown when the animals were placed in constant darkness [5]. Concerning interictal discharges it is the influence of sleep and wake that has been studied instead of circadian rhythmicity [6] and no data on clock gene profiles in epilepsy patients has been published. More is known about the influence of human epilepsy and seizures on circadian rhythms. One study has focused on chronotypes and found significant differences between patients with temporal lobe epilepsy (TLE) and juvenile myoclonus epilepsy [7]. In contrast, knowledge about core body temperature in epilepsy patients is minimal. Reduced heart rate variability and changed hormone levels, which are under the influence of the biological clock, have been observed in people with epilepsy [2].

If an interaction between circadian rhythm and epilepsy exists, further details may be of value for better understanding of the pathophysiology of epilepsy and the timing of diagnostic procedures and therapy. Therefore, this interaction is the focus of broad research in our and collaborating centres.

METHODS

Superficial EEG monitoring (s-EEG)

From 2003 to 2007, EEGs of all consecutive patients with clinico-electrographic seizures during continuous EEG and video recordings of ≥ 22 hours in our epilepsy centre were included. Recordings were performed using the 10-20 system with additional electrodes F9, F10, P9 and P10, according to the 10-10 system. Submental electromyography (EMG), electro-oculography (EOG) and electrocardiography (ECG) electrodes were also used. Respiration was measured by abdominal piezo respiratory effort sensors.

Intracranial EEG monitoring (IEM)

Longterm IEM and video monitoring was performed at the University Medical Centre Utrecht. Subdural strips and grids were used, positioned depending on the suspected foci in the patient. Additional scalp EEG electrodes were placed according to the 10-20 or 10-10 system. Furthermore, simultaneous polygraphy (EMG, EOG etc.) and ECG was performed and respiration was monitored. From 1999 to 2008, intracranial EEGs (electrocorticography,

ECoG) of 33 consecutive patients with spontaneous seizures (electroencephalographic epileptic events ≥ five seconds) during IEM were included.

Data analysis
Entire s-EEG and ECoG recordings were evaluated by well trained technicians of the Clinical Neurophysiology department and by board certified clinical neurophysiologists. From the s-EEG and IEM data, time of seizure occurrence and lobe of origin were determined. To study temporal seizure distribution, the 24h day was divided into four bins of six hours. With the expected nadir of the core body temperature as starting point, the time bins covered the periods of 0500-1100h, 1100-1700h, 1700-2300h and 2300-0500h.
The non-parametric binomial test was used to test whether seizure numbers in the four bins were significantly different. This test measures differences between the expected percentages (25% per bin) and found percentages.

Chronotypes in epilepsy
Self-reported data on morningness-eveningness of patients with TLE and frontal lobe epilepsy (FLE) treated in our centre were analyzed. The epilepsy diagnosis, history, medication and comorbidity could be enlisted from the patient's file. Patients with significant psychiatric comorbidity or learning disabilities were excluded. To determine chronotypes, the validated Dutch version of the Morningness-Eveningness Questionnaire (MEQ) was used. This questionnaire has been developed by Horne and Ostberg and differentiates morning and evening types [8]. The MEQs were processed according to scoring lists. Patients were matched for gender and age (≤ 4 years age difference). Chronotypes of patients with TLE and FLE were compared to chronotypes of healthy controls (employees of our centre) and to each other, using the independent two-sample t-test (after verifying normal distribution with the Kolmogorov-Smirnov test).

In all three studies significance was set at p level <0.05. For statistical analysis SPSS v12.0.1 was used.

RESULTS AND DISCUSSION

Temporal lobe
We have included 65 adults (16-65y) with 241 clinical seizures and 13 children (1-15y) with 67 seizures of temporal origin in the s-EEG group. In the ECoG-group six patients had 85 seizures of mesial temporal origin (five adults, one child) and eight from neocortical temporal origin (n=72, seven adults).
In the s-EEG-group significantly more seizures of temporal origin in adults were observed in the time period 1100-1700h (p<0.001, binomial test). In the group of children the number of temporal seizures was smaller, although a trend can be seen with more seizures from 0500-1700h (p=0.056). Significantly fewer temporal seizures were seen from 2300-0500h in children and adults (p=0.016 and p<0.001, Figure 1). Likewise, in the ECoG-group a peak was found in seizures of mesial temporal origin from 1100-1700h (p=0.002) and fewer seizures were found from 0500-1100h (p=0.005). A similar timed peak (1100-1700h) was found in seizures from neocortical temporal origin (p=0.07) (Figure 2).
Chronotypes of 25 TLE patients (12F; mean age 39y [17-56]) were assessed by the MEQ. When comparing these chronotypes to controls (12F; mean age 39y [17-58]) no differences were found (p=0.15, independent two-sample t-test).

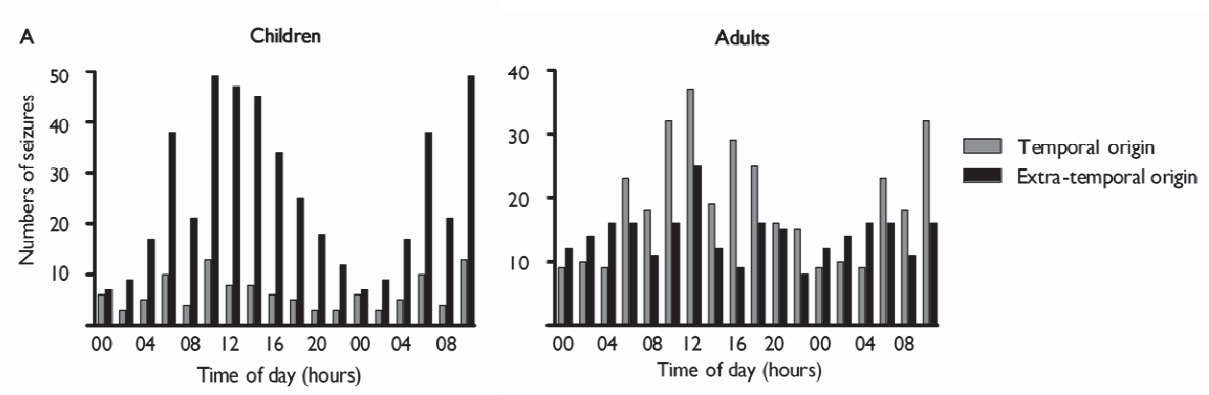

Figure 1. Bar histogram showing temporal distribution of seizures of temporal and extra-temporal origin in more detail in children (A) and adults (B). Each bar represents numbers of seizures per 2h. Data of 36h is given to show 24h cosinor rhythmicity [9].

Extra-temporal lobe

Thirty five adults with clinical seizures of extra-temporal origin (n=171) were included in the s-EEG-group, together with 63 children (n=329). In the ECoG-group 190 frontal seizures were seen in 14 patients (ten adults), 99 seizures from parietal origin in four patients (three adults) and four occipital seizures in one adult.

In the s-EEG-group significantly more seizures of extra-temporal origin in children were observed from 1100-1700h (p<0.001, binomial test) and significantly fewer from 2300-0500h (p<0.001). In adults a same trend can be seen, however, this did not reach significance (Figure 1).

In the ECoG-group more frontal seizures were seen during night time (2300-0500h, p=0.049). Seizure occurrence from parietal origin peaked from 1700-2300h (p=0.008) and seizures were less prevalent from 1100-1700h (p=0.024, Figure 2). Due to scarce data, occipital seizures were excluded from analysis.

Also, chronotypes of 25 patients with FLE (12F; mean age 37y [18-63]) were assessed by the MEQ and compared to controls (12F; mean age 38y [17-63]). No differences in chronotypes were found (p=0.09, independent two-sample t-test). Also when we compared 25 matched TLE-FLE pairs, no differences were observed (p=0.22).

CONCLUSIONS

We have shown that there is diurnal rhythmicity in human seizures. These patterns vary depending on the lobe of origin, with differences in rhythmicity between the temporal and extra-temporal brain areas. Within the extra-temporal area, strong differences were found with intracranial recordings. In addition, no differences in chronotypes between people with TLE and FLE and people without epilepsy were observed.

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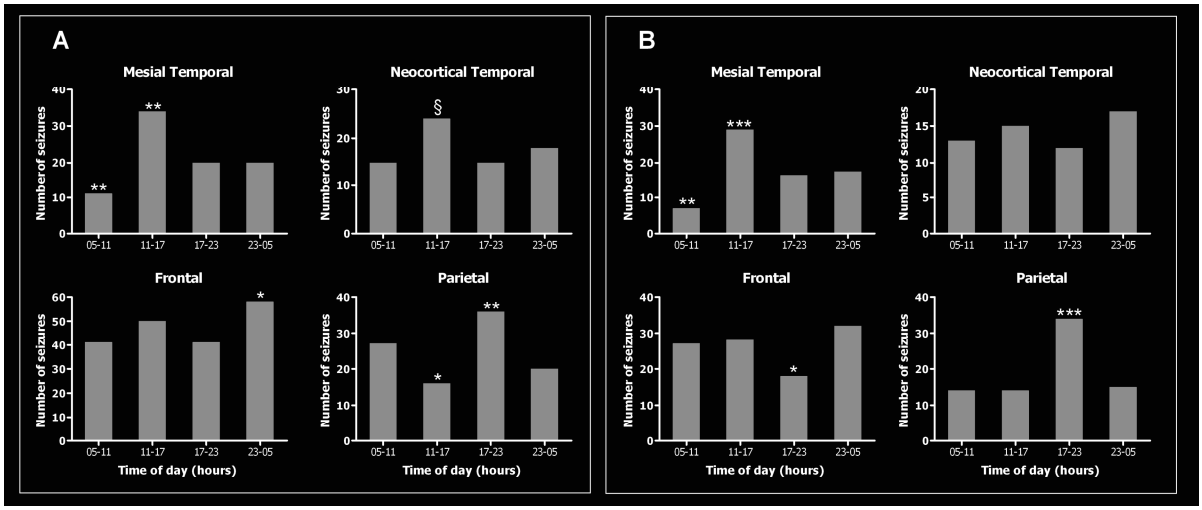

Figure 2. Bar histogram showing temporal distribution of seizures from different regions of the brain in children and adults (A, n=446) and adults only (B, n=308). Each bar represents seizure numbers per time bin of six hours (* $p<0.05$; ** $p<0.01$; § $p=0.07$; *** $p<0.001$) [10].

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QUALITY OF LIFE AND SLEEP DISTURBANCES OF DAYTIME HEMODIALYSIS PATIENTS

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INTRODUCTION

End-stage renal disease (ESRD) is associated with an increased prevalence of sleep disturbances [1]. Approximately 50-80% of patients with ESRD complain about disturbances of the sleep-wake rhythm [1]. In this patient group circadian rhythmicity, defining periods of low and high sleep propensity [2], can be negatively affected due to pathology of ESRD and the dialysis process, causing daytime sleepiness and insomnia [3]. In the general population nocturnal and daytime sleep abnormalities adversely affect quality of life-related measures [4]. Thus far, only one study with 46 ESRD patients has recorded objective sleep characteristics (with polysomnography), in addition to subjective sleep and quality of life [5]. A negative correlation between periodic limb movement index and health and functioning was found.
The present study is the first to study the relationship between objective sleep (by means of actigraphy), daytime sleepiness (by means of the Epworth Sleepiness Scale, = ESS) and quality of life (by means of the MOS SF-36) in a larger group of hemodialysis patients.

METHODS

This paper reflects the baseline characteristics of the MELODY study, which is a randomized double-blind placebo-controlled study of 1 year with melatonin 3 mg. The Medical-Ethical Committee approved the protocol of the study (ClinicalTrials.gov: NCT00388661), and informed consent was obtained from all patients. Patients between 18 and 85 years and on stable hemodialysis (more than 3 months on hemodialysis with an adequate dialysis efficacy) were included. If the patients had a regime of Monday-Wednesday-Friday haemodialysis all measurements during the study were started on a Monday. If patients had a regime of Tuesday-Thursday-Saturday hemodialysis all measurements were started on a Tuesday. At baseline, daytime hemodialysis patients were asked to fill out a sleep questionnaire and to wear an actometer for seven consecutive days.
During treatment, vascular access was achieved by 2 needle placements in fistulas or grafts or by internal jugular catheter. Dialysate flow was kept at 300 ml/min, blood flow was set at 200-300 ml/min.
Model Actiwatch-L (Cambridge Neurotechnology Ltd ®, Cambridge, United Kingdom) were used. The actometer is placed on the wrist of the arm without a graft or fistula. The following

parameters were calculated, according to standardized methods [6]: Actual Sleep Time, Actual Awake Time, Sleep efficiency, Sleep Onset Latency and Fragmentation Index. Fragmentation Index is the total number of wake bouts (defined as a wake period by the algorithm of the software) divided by the total sleep time in hours.
The questionnaire used for measuring physical, functional, mental and social health was the Dutch version of the RAND-36 [7].The scores of the hemodialysis patients were compared with the Dutch population above 65 years [7].
Sleepiness was assessed by means of Epworth Sleepiness Scale (ESS). The range of possible scores on the ESS is 0-24, with higher scores indicating greater levels of subjective sleepiness. A score > 9 is often used to identify individuals with sleep disturbances [8]. Mean and standard deviations of the actigraphy, sleep questionnaire and quality of life measures were calculated. Pearson correlation analysis was performed. The Student T-test was used to find significant differences between values (P-values < 0.05 were considered to represent statistical significance).
The Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 17 was employed for all statistical analysis.

RESULTS AND DISCUSSION

The general characteristics of the patients included are shown in Table 1. As can be seen, these characteristics are matched for a normal hemodialysis population in the Netherlands [9]. In the group 42 patients were male and 28 patients were female.

Table 1. General characteristics of the patient group, displayed as mean and standard deviation.

Parameters	*Mean [sd]*
Age (in years)	65 [25]
Kt/V pro week incl. residual kidney function*	4.1 [0.7]
BMI (kg/m2)	26 [7]
Dialysis duration per week (in hours)	11.2 [2.0]

* Kt/V: Index of dialysis adequacy, fractional reduction of urea [10]

Table 2 shows that both on the night following hemodialysis treatment and the consequent night without daytime hemodialysis most sleep parameters are impaired. Especially SE ($p < 0.01$) and AAT ($p < 0.01$) were significantly impaired in comparison to normal values.
Patients scored a mean of 11.5 points (SD ± 5) on the ESS questionnaire.

Table 2: Results of the actometer on the night with daytime dialysis and the following night without daytime dialysis (displayed as mean and standard deviation) (n= 70)

Parameters	*Normal values [11,12]*	*Mean [sd] Night with daytime hemodialysis*	*Mean [sd] Following Night without daytime hemodialysis*
Sleep onset latency (min.)	< 30	37	32
Sleep efficiency (%)	> 85	71*	68*
Actual Awake time (%)	< 10	22*	24*
Actual Sleep Time (min.)	> 350	359	329
Fragmentation Index	#	8	8

Normal values not known yet, * $p < 0.05$

Table 3 shows the results from our hemodialysis population and the measures extracted from the general Dutch population. For nearly all scales the patient group obtained lower scores than the control subjects.

Table 3: RAND-36 (n = 70)

Parameters	*Normal values Subjects (> 65 years)*	*Patients; Mean values*	*P*
Physical functioning	66.7	43	<0.01
Social functioning	83.2	55	<0.01
Role disability due to physical problems	69.1	34	<0.01
Role disability due to emotional problems	82.9	56	<0.01
Mental health	75.9	69	0.02
Vitality	64.2	48	< 0.01
Bodily pain	74.8	60	<0.01
General health	60.1	36	<0.01
Health change	46.8	52	>0.05

Correlations between subjective sleep (measured with ESS) and objective measurements by means of actigraphy regarding sleep efficiency ($r = - 0.41$, $p < 0.01$), actual sleep time ($r = - 0.42$, $p < 0.01$), and awake time ($r = 0.32$, $p = 0.01$) were significant. A trend was found concerning sleep onset latency ($r = 0.22$, $p = 0.08$) and fragmentation index ($r = 0.23$, $p = 0.06$). No significant relationships were found regarding actigraphy results and RAND-36 scales, Furthermore, no correlations were found between subjective sleep and RAND-36 scales. When comparing quality of life between 'good and 'bad' sleepers, general health was significantly lower in the 'bad' sleepers group (ESS score above 9) in comparison to the 'good' sleepers ($p = 0.02$).

As correlations between quality of life and sleep parameters were not found, it can be hypothesized that sleep quality is only a minor contributor to their general quality of life. In Delayed Sleep Phase Syndrome (DSPS) patients, who primarily have sleep onset problems, sleep quality was highly correlated to quality of life [11]. ESRD patients suffer from severe and multiple morbidities, such as an increased prevalence of cardiovascular disease [12]. It is possible that the RAND-36 scales are not discriminately enough to correlate sleep and quality of life in this patient group. Other explanations can be that the sample size is too

small or that the RAND SF-36 does not assess sleep quality or sleepiness, which seems to be an omission of the questionnaire.

CONCLUSIONS

The results show that ESRD patients on daytime hemodialysis experience sleep disturbances, as subjective and objective measured sleep were both impaired. Furthermore, their quality of life is decreased in comparison to the general population. We did not find the expected correlations between sleep and quality of life. The possible explanations could be the multiple problems of ESRD patients and the poor discriminator measurements of quality of life by means of MOS-SF 36. More research on quality of life measurements in ESRD, such as the outcomes of the MELODY study, is warranted to confirm these explanations.

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COMPARISON OF PERSONALITY TRAITS IN INSOMNIACS AND PATIENTS WITH OTHER KIND OF SLEEP DISORDERS

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INTRODUCTION

Patients who visit a sleep center for consultation and treatment usually will be seen by the medical specialist in the first place. Although in all cases the main problem is disturbances of sleep or wakefulness, clinicians seem to interact in a different way in their own interaction insomnia patients and patients with any other sleep disturbances. Differences in personality characteristics of these groups of patients might play a role in the clinicians' attitude. Studies already have indicated that patients with insomnia exhibit more symptoms of depression and stress when compared with normal sleepers[1,2]. Moreover they show an increased need of being in control, as indicated by several scales of the Minnesota Multiphasic Personality Inventory (MMPI)[3-5]. Relatively little is known about personality traits in patients with OSAS, PLMD or RLS. Up to now, results indicate some psychopathological symptoms on the MMPI in these patients[6], with PLMD patients being more likely to report psychopathological symptoms than OSAS patients[7]. By integrating the scores of several tests that pretend to measure personality characteristics, a statement on the personality traits can be made. Our study therefore aims at examining whether there is a difference between personality traits in insomnia patients and patients with other kind of sleep disorders. The focus is on the coping style, ruminating behavior and their presentation of the complaints. If differences are found, they may explain the difference in the perception by the clinicians, and should be taken into account in the communication with patients in that case.

METHODS

Twohundred-and-fifty-nine patients, 151 men and 108 women, of the Center for Sleep- Wake Disorders, MCH Westeinde Hospital, The Hague, participated in this study. They all had chronic sleep complaints (> 1 year). Polysomnography was conducted over app. 48 hours at the patient's home. On both days of these PSG-recordings, they filled out the Groningen Sleep Quality Scale (GSKS) to assess the subjective sleep quality of the night before. At one day the questionnaires SCL-90 (Symptom Checklist) and PSWQ (Penn State Worry Questionnaire) were added and at the other day the UCL (Utrecht Coping List) and NPV (Dutch Personality Questionnaire). All questionnaires are well-validated Dutch versions that have shown reasonable to good reliability. Individuals had to be 16 years or older and their sleep disorder existed for at least one year. Exclusion criteria were: severe psychiatric disorder and a poor control of the Dutch language. After filling out the questionnaires and after the final diagnosis 21 subjects were excluded because of reasons like not having any sleep disorder at all or failure to proceed the questionnaires properly.

Fourty-two percent of the cases were diagnosed having insomnia, with a majority of women in this group (68,6%). The sleep disorders in the other patients (Other) were mainly sleep related breathing disorders (34,7%) and PLMD / RLS (10,4%), mainly men. The data of the questionnaires was analyzed by using the plain scores. Scale scores on the questionnaires of both groups were compared using independent-samples T-tests. The Levene's test was conducted to verify the assumption of equality of variances.

RESULTS AND INTERPRETATION

On the personality questionnaire, NPV, the insomnia group has significant lower scores on the scales Hostility, Egoism, Dominance and Rigidity. Insomnia patients also tend to score higher on Neuroticism. See table 1.

Table 1. Independent T- test: mean ± SD of both groups using the NPV

	Insomnia	*Other*	*t*	*p*
Neuroticism	14,48 (8,75)	12,57 (8,55)	1,70	n.s.
Social anxiety	8,95 (7,04)	10,09 (7,87)	1,16	n.s.
Rigidity	23,73 (8,92)	27,27 (8,26)	3,18	0,002
Hostility	13,59 (6,60)	18,05 (7,70)	4,74	0,000
Egoism	9,36 (4,48)	12,44 (5,75)	4,66	0,000
Dominance	13,93 (6,10)	17,02 (6,90)	3,62	0,000
Self-esteem	24,38 (7,23)	25,32 (7,45)	0,98	n.s.

n.s. = not significant (p>0.05)

According to the results on the UCL (coping list), insomnia patients tend to seek social support in difficult situations or in times of problems more than the other group. They also show a higher degree of passive coping and palliative reaction. See table 2.

Table 2. Independent T- test: mean ± SD of both groups using the UCL

	Insomnia	*Other*	*t*	*p*
Active coping	18,78 (3,82)	19,04 (3,71)	0,53	n.s.
Palliative reaction	18,26 (3,43)	17,36 (3,40)	2,05	0,042
Avoidance	16,37 (2,99)	16,17 (3,32)	0,49	n.s.
Seeking social support	14,34 (3,52)	12,73 (3,51)	3,55	0,000
Passive coping	13,10 (3,84)	12,11 (3,39)	2,14	0,033
Expression of emotions	6,51 (1,49)	6,33 (1,64)	0,91	n.s.
Reassuring thoughts	12,11 (2,39)	12,10 (2,58)	0,02	n.s.

n.s. = not significant (p>0.05)

The results on the SCL-90 indicate that insomnia patients perceive more sleep problems than patients with other kind of sleep disorders. This finding was confirmed by the GSKS outcome with a lower total score for the insomnia patients (t=4,9; p< 0,001), indicating that during the registration sessions the sleep was perceived worse by insomnia patients as well. Besides that, they have more complaints of depressive and anxious feelings. See table 3.

Table 3. Independent T- test: mean ± SD of both groups using the SCL-90

	Insomnia	*Other*	*t*	*p*
Anxiety	8,90 (3,70)	8,68 (2,55)	0,53	n.s.
Phobic complaints	17,36 (6,54)	15,58 (5,29)	2,33	0,021
Depression	29,78 (10,43)	26,92 (8,66)	2,31	0,022
Somatic complaints	23,09 (8,35)	23,16 (7,77)	0,07	n.s.
Obsessive	20,61 (7,73)	19,22 (6,40)	1,52	n.s.
Sensitivity	27,81 (9,51)	27,09 (11,68)	0,52	n.s.
Hostility	8,56 (2,69)	8,67 (3,23)	0,29	n.s.
Sleeping problems	10,57 (3,09)	7,28 (3,28)	7,94	0,000
Psychoneuroticism	157,95 (44,20)	147,38 (39,96)	1,94	n.s.

n.s. = not significant (p>0.05)

Table 4. Independent T- test: mean ± SD of both groups using the PSWQ

	Insomnia	*Other*	*t*	*p*
PSQW	48,75 (13,31)	44,35 (11,79)	2,71	0,007

According to the PSWQ (worrying scale), insomnia patients tend to worry more than the other sleep disordered patients. See table 4.

Parts of the results are comparable with the outcomes found in studies where personality traits of insomnia patients were compared with personality traits of normal healthy subjects. Their lower self esteem and self-confidence and their tendency to react in a more passive way are already known (8). In literature we did not find the high degree of tolerance patients with insomnia claim to have with respect to others (NPV, Hostility). Tolerance and patience in itself can be seen as positive characteristics. These characteristics get another loading when seen against the insomniacs' neurotic fear of losing the love and appreciation of the other person. The neurotic fear is the engine of their adjustment to wishes of their environment, although the insomniac will neither experience this fear, nor experience his adjustment in this way.
According to their coping style, insomnia patients tend to seek comfort, appreciation and other kinds of social support when having problems. This finding is in accordance with their lower self-esteem and their need for affection. Excessive use of sleep medicine (31% of the insomniacs takes at least two times a week sleeping pills while this is 8% in the Other group; not published data from this study) can be seen as an expression of their tendency to show a more palliative reaction style compared with the Others.

DISCUSSION

Although clear differences between the two groups of patients were found, one should realize that the sleep disorders are heterogeneous in both groups. Psychophysiological and paradoxical insomnia, mere bad sleep hygiene and other kinds of insomnia were represented in the insomnia group; in the other group mainly sleep related breathing disorders, PLMD, RLS and to a lesser extend some other disorders. Nevertheless both groups were similar in having a sleep disorder. Although not given in detail, the differences in sleep parameters were also analysed. Comparisons with independent t-tests indicated no differences in important sleep characteristics in the night's sleep, as measured with polysomnography. Sleep efficiency, time in bed, total sleep time, sleep onset latency and amount of slow wave

sleep were comparable for both groups. It is therefore assumed that sleep itself is not the (main) cause of the complaints of both patient groups. On the other hand, more naps during the day were found in the Others group, and as a consequence it could be stated that the Others might get more sleep during 24 hours. Whether or not the differences in naps can completely explain the differences in personality, merits further studies.

CONCLUSION

Insomnia patients show more neurotic symptoms compared to the group with other sleep disorders,. They are less content about their own person, and their complaints are depressive in nature. Furthermore, their level of anxiousness is higher. The various scores indicate a more subassertive and submissive behavior, and more suppression and more internalization like worrying. The latter is more general and not only related to sleep. In addition, insomniacs tend to take their cue from others: they are open to other people; they are eager to please and want to show their empathy. They prefer to be treated by others in the same way. Probably this is what makes them more vulnerable in their interaction with other people and this will also have some influence on their (nonverbal) communication. It can be assumed that the differences in personality characteristics between the insomniacs and people with other kinds of sleep problems contribute to the clinicians' perceived differences in their interaction with both groups and the clinicians' attitude.

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EFFECT OF HYPOXIA/REOXYGENATION ON NEUTROPHIL FUNCTION: A PUTATIVE IMPORTANT SOURCE OF OXIDATIVE STRESS IN THE SLEEP APNOEA SYNDROME?

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INTRODUCTION

The higher mortality of patients with obstructive sleep apnoea (OSA) is related to a higher incidence of obesity, metabolic syndrome, diabetes and cardiovascular disease in these patients.[1] The pathophysiological mechanisms mediating these associations have not been fully elucidated. There is however increasing evidence that oxidative stress is the linking factor between OSA and cardiovascular disease.[2] The oxygen desaturation (and reoxygenation) can be a trigger for polymorphonuclear neutrophils (PMNs) to be activated, thereby releasing free oxygen radicals as an inflammatory response. Basically, oxidative stress is defined as an imbalance between oxidant producing systems and anti-oxidant defense mechanisms, resulting in excessive formation of reactive oxygen species (ROS), also called respiratory burst.[3] The aim of the present study was to investigate the role of oxidative stress by monitoring the impact of episodes of hypoxia and re-oxygenation on the function of the PMNs in an in vitro experiment. Function was assessed with respiratory burst, elastase secretion and intracellular redox status. When PMNs are activated, it is associated with an increase in elastase. Therefore, the concentration of elastase released in the supernatans can be used as a measure of inflammation. Moreover, the intracellular redox status (reduced and oxidised glutathione) was measured to reflect the antioxidant capacity.

METHODS

Human polymorphonuclear (PMN) cells isolated from peripheral blood out of 7 healthy subjects were either incubated overnight or used directly after sampling. They were exposed for 3 hours to either normoxia (N:18% O_2), hypoxia (H:1% O_2) or hypoxia (1% O_2, 3hours) followed by normoxia (18% O_2, 30 min) (H/N). Function was then assessed by monitoring respiratory burst (luminal-enhanced chemiluminescence) with two stimulants (PMA and fMLP, used as stimulator to provoke the respiratory burst "in vitro"). The intensity of respiratory burst can be expressed with the mean RLU (relative luminescence units), the integral and the maximal measured value. Moroever, elastase secretion induced by fMLP or PBS^+ and total and oxidised glutathione was measured with spectrophotometry.

A first series of experiments was performed on PMN which were incubated overnight on normoxic conditions (old cells), and which were then exposed to hypoxia and

hypoxia/reoxygenation. A second series of experiments took place on fresh isolated cells. Before incubation of the hypoxic cells took place at night, equilibration of the medium was performed with an hypoxic gas mixture in a nitrogen box (5% CO_2, 1% O_2, 94% N_2). After equilibration cells were added in a concentration of 20 million PMN/20 ml medium. The time the cells passed in the different conditions is shown in Table 1.

Table 1. The time the polymorphonuclear cells passed in the different conditions.

Condition	*Time (h) in hypoxia*	*Time (h) in normoxia*
Normoxia	0	2.5
Hypoxia	3	0
Hypoxia/normoxia	3	0.5

The first 30 minutes were used to equilibrate the medium with the cells. Therefore, the effective time in hypoxia was estimated 2.5 hours.

RESULTS AND DISCUSSION

First of all, cell viability was determined after the overnight incubation and after the experimental procedure. Viability was above 90% in the old as well as in the fresh cells. However, there was a loss in number, since 25% of the cells were lost.

Respiratory burst was analysed based on a comparison between the integral of the measurement and the peak RLU. The integral was significantly higher for fresh cells compared to old cells, with PMA as well as fMLP. Respiratory burst induced by fMLP was higher in fresh neutrophils after exposure to H and H/N (1375±593 and 1323±508 versus 614±368 integrated RLU over 60 min in N, $p = 0.03$ and $p = 0.009$ respectively). When PMA was used as a stimulans, no significant differences were noticed in the different conditions (Figure 1).

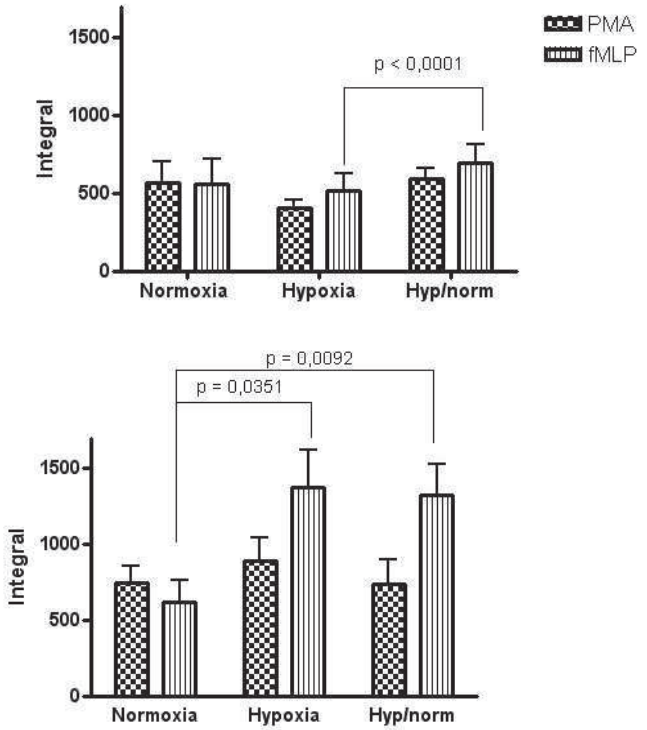

Figure 1. Integral of respiratory burst (RLU * 60 minutes) in different study conditions with Old cells (left), and Fresh cells (right). Results as mean ± SD.

Elastase release was also significantly increased after induction by fMLP (1398±1563 after H and 3746±4136 after H/N versus 217±183 µU/ml after N, p = 0.03 and 0.007 respectively.). These increases were not detected after induction by PMA or in the cells which had been incubated overnight before exposure to H or H/N (Figure 2).

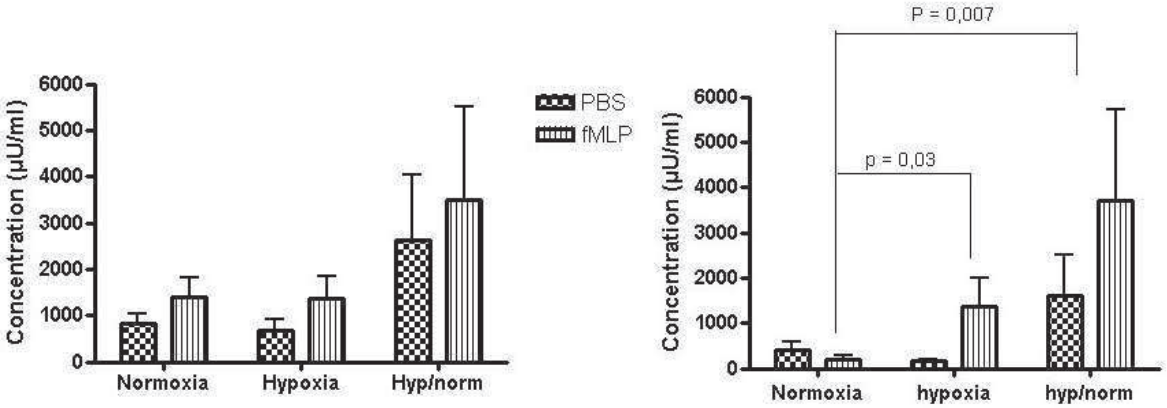

Figure 2. Elastase concentration in different study conditions with Old cells (left), and Fresh cells (right). Results as mean ± SD.

Fresh cells had a significantly higher concentrations of total glutathione, which did not differ according to the different oxygenation conditions. Reduced glutathione content was higher

in fresh cells (p = 0.0087) but oxidised glutathione did not differ. Neither reduced nor oxidised glutathione levels were affected by exposure to different O_2 conditions (Figure 3).

No significant differences (p=0.75) could be found in the concentration of oxidised glutathione between old and fresh cells, nor between the different oxygenation conditions (not shown). A significant difference (p=0.03) was observed in the ratio total glutathione/GSSG between old and fresh cells, with higher values in fresh cells. Since no difference was observed in GSSG concentrations between old and fresh cells, this difference must be due to a difference in concentration of total glutathione (Figure 4).

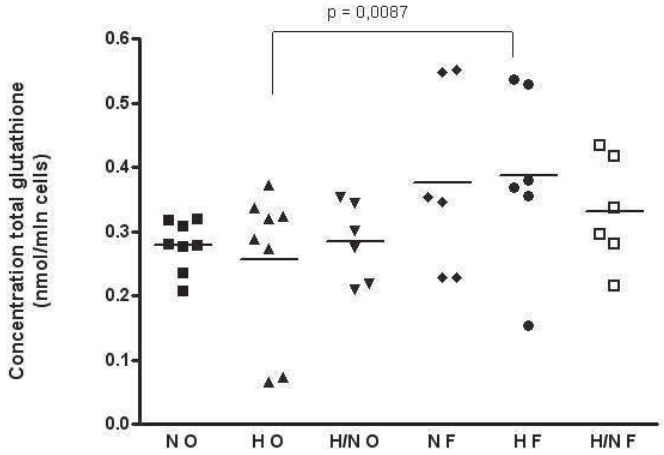

Figure 3. Concentration of total glutathione in PMN exposed to different study conditions (O: old cells, F: fresh cells)

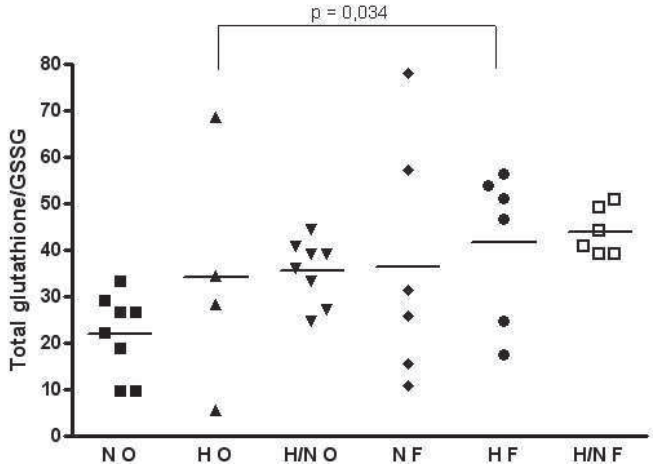

Figure 4. The ration of total glutathione/GSSG in PMN exposed to different study conditions (O: old cells, F: fresh cells)

Hypoxia and hypoxia/reoxygenation promote the secretion of reactive oxygen species and proteolytic enzymes by neutrophils. This confirms that intermittent hypoxia may be an important source of the increased oxidative stress observed in OSA and may contribute to the higher cardiovascular morbidity and mortality in these patients. This study showed that the effects of H/N are strongly dependent of the state of the cells, with positive results (respiratory burst, increase in elastase, and increase in reduced glutathione) in fresh cells

only. Hence, studies on oxidative stress have, as a consequence, to be performed in fresh cells.
We demonstrated that reduced glutathione and the ratio total glutathione/GSSG is increased in fresh cells. This indicates an activation of the antioxidant capacity, since glutathione is one of the most important antioxidants. This study also demonstrates that it is possible to measure glutathione, which is considered as a very unstable and volatile molecule.
The impact of H/N on the redox state and on the function of PMN, compared to H alone, is limited. This is an unexpected finding, since oxidative stress develops after reoxygenation occurs. This effect could be explained by a potential artefact in the method, like exposure of the cells to room air after hypoxia, which could partially lead to reoxygenation.

CONCLUSIONS

Hypoxia/reoxygenation can lead to an activation of neutrophil function, with increased respiratory burst, elastase release and activation of antioxidant capacity. Although these experiments were performed in an in vitro model, the findings suggest that this PMNs activation is a putative important source of oxidative stress in OSA, contributing to the development of cardiovascular disease. This model confirms earlier clinical observations.

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REDOX STATUS AND NEUTROPHIL FUNCTION IN THE SLEEP APNOEA SYNDROME

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INTRODUCTION

In an earlier contribution we could demonstrate that exposure to different hypoxic conditions could lead to an enhanced respiratory burst in polymorphonuclear cells (PMN), after stimulation with fMLP (N-formyl-L-methionyl-L-leucyl-L-phenylalanine).[1] fMLP is used as a stimulator to provoke the respiratory burst "in vitro".
The aim of the present study was to investigate oxidative stress in patients with obstructive sleep apnoea, by monitoring respiratory burst, and measurement of total and oxidised glutathione. Moreover, elastase was determined as marker of activation of PMN.[2]

METHODS

A number of consecutive patients, referred to the Sleep Disorders Center of the Antwerp University Hospital due to suspicion of sleep related breathing disorder, were included. We used the following exclusion criteria: COPD, diabetes, pregnancy, chronic fatigue syndrome, neuromuscular disorders, younger than 25 years, use of substance with an antioxidant effect or anti-inflammatory effect (vitamins, minerals, glucocorticosteroids, antibiotics, acetylcysteine), thyroid diseases, recent surgery and common cold at the moment of the evaluation. After informed consent a fasting blood sample (3*10cc Li-Heparine) was taken in the morning, close after awakening. 1 tube was aimed to perform measurements in plasma and full blood. 200µl were used to determine Hemoglobine concentration and reduced glutathione concentration. This blood sample was centrifugated during 10 minutes on 3000 rpm at 4°C without inhibition. Obtained plasma was frozen at -20°C to determine elastase. 500 µl was used to determine total protein and SH-groups. The two other tubes were used to characterized the polymorphonuclear and mononuclear cells. Function was then assessed by monitoring 1) respiratory burst (luminal-enhanced chemiluminescence) with two stimulants (PMA and fMLP), 2) elastase secretion (spectrophotometry) induced by fMLP or PBS^+ and 3) intracellular redox status (reduced and oxidised glutathion by spectrophotometry).
After analysis patients were divided into 2 groups: Group 1 had nonapneic snoring or mild OSA (AHI<15), Group 2 had moderate-to-severe OSA (AHI > 15). Measurements were performed in 2005.

Measurement of respiratory burst by chemiluminescence: during the respiratory burst of PMN a release of reactive oxygen species (ROS) occurs, like superoxide radicals ($O2^{\cdot}$), hydrogenperoxide (H_2O_2) and peroxyl radicals ($ROO^{\cdot}$). When luminol is added, it is oxidised by the different radicals and converted to an excited condition. This excited aminophtalate anion emits photons when it relaxes to basic conditions. The resulting light

emission is a measure of ROS concentration. PMA (Phorbol 12-Myristate 13-acetate) and fMLP (N-formyl-L-methionyl-L-leucyl-L-phenylalanine) are used as stimulator to provoke the respiratory burst "in vitro". The intensity of respiratory burst is expressed with the mean RLU (relative luminescence units), the integral and the maximal measured value.
Measurement of elastase: when PMN are activated, it is associated with an increase in elastase. The concentration of elastase released in the supernatans is a measure of inflammation. To measure the concentration of released elastase, a chromogene peptide substrate is used (N-methoxy-succinyl-ala-ala-pro-val-p-nitroanilide) at 405 nm. The quantity of p-nitroanilide released by PMN can be measured and its concentration is calculated with a calibration line prepared from a commercial elastase kit. The PMN were first incubated with fMLP (10^{-7} M) and PBS^{+} (phosphate buffered saline, with Ca^{2+} and Mg^{2}) during 1 hour on 37°C.

Measurement of total and oxidised glutathione by spectrophotometry: the method is based on a recycling mechanism between oxidised and reduced glutathione. The present oxidised glutathione is converted to reduced glutathione by NADPH and GSH reductase. Reduced glutathione reacts with DTNB (5,5' dithiobis-2-nitrobenzeen acid), which leads to the production of oxidised glutathione and a yellow chromophore TNB. TNB can be measured at 412 nm. The response time (slope as maxOD/min) is linearly correlated with the intracellular glutathione concentration. After creating a calibration line with thinned glutathione, the concentration of reduced glutathione can be calculated by linear regression. When 2-vinylpyridine is added to remove reduced glutathione, oxidised glutathione can be measured. The ratio total glutathione/GSSG can then be calculated.

RESULTS AND DISCUSSION

6 patients were evaluated in Group 1 and 11 patients in Group 2. The characteristics of the study groups are shown in Table 1.

Table 1. Patients characteristics in the two study groups

	AHI<15	*AHI≥15*	*p*
N	6	11	
Age (yrs)	46±11	48±14	NS
BMI (kg/m^2)	31±2	31±7	NS
Neck circumference (cm)	41±4	44±3	NS
BP systolic (mmHg)	132±28	129±28	NS
BP diastolic (mmHg)	84±17	78±6	NS
AHI (#/h)	8±4	31±24	0.01
SaO_2>90% (%)	97±3	90±13	NS
SaO_2<88% (min)	5±6	10±10	NS

(Mann-Whitney U test)

The parameters of oxidant status and leucocyte function are shown in Table 2 and 3.

Table 2. Monocyte respiratory burst, elastase activity and redox status in the study groups

	AHI<15	*AHI≥15*	*p*
Total protein (g/dl)	72±6	76±8	NS
µmol SH/g protein	398±119	336±103	NS
Plasma elastase (µU/ml)	43389±10000	41016±6949	NS
GSH (µmol/g Hb)	4.6±2.1	4±1	NS
Monocyte elastase PBS (µU/ml)	11864±1186	46101±53220	NS
Monocyte elastase fMLP (µU/ml)	15085±1525	23729±26949	NS
Monocyte RB fMLP	201±353	70±36	NS
Monocyte RB PMA	66±52	64±34	NS
Monocyte GSSG (nmol/10^6 cells)	0.0066±0.0061	0.0032±0.0026	NS
Monocyte Total glutathione (nmol/10^6 cells)	0.327±0.277	0.401±0.251	NS
Monocyte ratio	73±10	509±681	NS

(Elastase released by monocytes; monocyte RB: integral of respiratory burst of mononuclear cells after stimulation with PMA or fMLP; monocyte ratio: ratio total glutathione/GSSG of mononuclear cells)

Table 3. Polymorphonuclear cell respiratory burst, elastase activity and redox status in the study groups

	AHI<15	*AHI≥15*	*p*
PMN elastase PBS (µU/ml)	84914±54576	43220±34576	NS
PMN elastase fMLP (µU/ml)	334404±282201	119829±105931	NS
PMN RB fMLP	1716±2293	1246±681	0.09
PMN RB PMA	558±383	776±355	0.08
PMN GSSG (nmol/10^6 cells)	0.0028±0.0017	0.0065±0.0049	NS
PMN Total glutathione (nmol/10^6 cells	0.372±0.519	0.589±0.185	0.03
PMN Ratio	230±209	262±385	NS

(Elastase released by PMN; PMN RB: integral of respiratory burst of PMN after stimulation with PMA or fMLP; PMN ratio: ratio total glutathione/GSSG of PMN)

The only statistically significant difference that could be observed was the concentration of total glutathione of the PMN. Total glutathione was higher in the group with moderate to severe OSA.

In the PMN cells, there was a trend in the patients with moderate to severe OSA to release less elastase compared to patients with mild OSA. This trend was present in stimulated as well as non stimulated cells.

We could demonstrate a significant correlation between total glutathione (PMN and mononuclear cells) with the integral of the respiratory burst, both after PMA and after fMLP as stimulant. The response of respiratory burst of mononuclear cells is however very low.

The correlation between total glutathione in PMN and SH protein and plasma elastase were also evaluated. Results are shown in Table 4.

Table 4. Correlation between total glutathione of PMN and mononuclear cells and RB fMLP, RB PMA, PMN elastase (PBS), PMN elastase (fMLP), SH protein and plasma elastase

PMN total glutathione	*RB fMLP*	*RB PMA*	*PMN elastase (PBS)*	*PMN elastase (fMLP)*	*SH protein*	*Plasma elastase*
Pearson Corr	0.54	0.57	-0.41	-0.42	-0.76	0.14
p	0.02	0.02	0.09	0.13	<0.01	0.67

(RB fMLP: integral of respiratory burst of PMN stimulated with fMLP; RB PMA: integral of respiratory burst of PMN stimulated with PMA; PMN elastase: elastase release in the supernatans of PMN non stimulated (PBS) or stimulated (fMLP); SH protein (µmol SH/g protein) and elastase in plasma)

CONCLUSIONS

The goal of this study was to evaluate the PMN as source of oxidative stress in OSA. We could not observe an increase in respiratory burst in PMN nor in monocytes in OSA AHI≥15. On the contrary, a trend to a lower respiratory burst was seen in PMN and monocytes after stimulation with fMLP. Moreover, PMN showed a trend to a lower release of elastase in AHI≥15, while monocyte release of elastase was increased. The only significant finding was an increase of PMN total glutathione, but this is hard to explain, since nocturnal glutathione consumption takes place in OSA and lower glutathione levels are expected, in line with findings in AIDS and cystic fibrosis.[3,4] We could demonstrate that glutathione can be measured intracellularly, which is a major contribution of this study. Although a healthy control group was lacking, we believe that our data are relevant, since an AHI of <15 is not associated with cardiovascular burden. A weakness of the study is however the rather low number of patients in both groups. More patients and a healthy control group could be useful to make definite conclusions.

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PULSE TRANSIT TIME (PTT): A USEFUL TECHNIQUE TO ASSESS CENTRAL APNEAS IN A ROUTINE SETTING.

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INTRODUCTION

It was reported before that Pulse transit time (PTT) is a useful non-invasive tool to measure the inspiratory effort in patients with sleep-related breathing disorders[1]. PTT is defined as the time the pulse wave needs to travel between two arterial sites, from the aortic valve to a peripheral site, f.i. the finger top. It can be measured with photoplethysmography (PPG), which makes use of an infrared oximeter probe, attached to the fingertop. PPG measures the change in the volume of red blood cells in the microvascular bed with each pulse. The PPT signal is created at each heart beat, from the R wave on the electrocardiogram to the pulse wave arrival at the finger. PTT fluctuations have been shown to correlate well with inspiratory effort against a threshold valve in awake normal volunteers and with inspiratory effort measurements based on esophageal pressure monitoring with an esophageal catheter (the gold standard method). It was proven before that PTT measurement during sleep is capable of discriminating central apneas and obstructive respiratory events with sufficient sensitivity and specificity. Therefore PTT can potentially become the reference non-invasive method for assessment of respiratory events during sleep[2].
Up to now, esophageal catheter measurements are considered the gold standard method for evaluation of respiratory effort, although this method is invasive and rather uncomfortable and may induce sleep disruption. However, thoracic and abdominal excursions are widely used in a routine setting to detect respiratory events and to distinguish between central and obstructive apneas. Adequate assessment of respiratory effort during sleep is extremely important to prevent misclassification of respiratory events and false diagnosis of central or obstructive apnea inherently leads to inappropriate treatment strategies. Overestimation of central apneas has been shown when measurements of respiratory effort are only based on strain gauges[3]. At present, respiratory inductance plethysmography (RIP) has become the recommended technique to monitor the nocturnal breathing pattern, but it's combination with PTT was not yet evaluated.
The aim of this study was to evaluate the accuracy of addition of PTT to respiratory inductance plethysmography combined with nasal canula and backup thermistors.

METHODS

Polysomnographies were performed in 12 patients referred for suspicion of sleep disordered breathing to our Sleep Disorders Centre (Antwerp University Hospital, Belgium). PTT was measured with a PPG (NONIN, Puresat®) based on the ECG channel with sample frequency of 1000 Hz. Sleep stages were manually scored according to standard criteria (Rechtschaffen and Kales). The polysomnographies were scored three times: based on the routine respiratory

signals, based on the PTT and finally, based on both signals. Events with complete absence of visibly detectable deflections for at least 10 sec in the thoraco-abdominal movement signals, were considered as central apnea. Events, for at least 10 sec, with a little interruption in the thoraco-abdominal movements were scored as mixed apneas. During the first step, PTT signal was made invisible on the sleep tracing. During the second step, routine respiratory signals were made invisible.
To finish, during the last step, all signals were made made visible and all apneas were rescored and definitely classified as central apnea when both PTT signal and thoraco-abdominal movement were absent. Also undetected central apneas, based on overlooked or unclear PTT signals were reanalysed based on both signals. A distinction was made between "false scored" central apneas, "true" scored central apneas and mixed apneas (Fig.1).

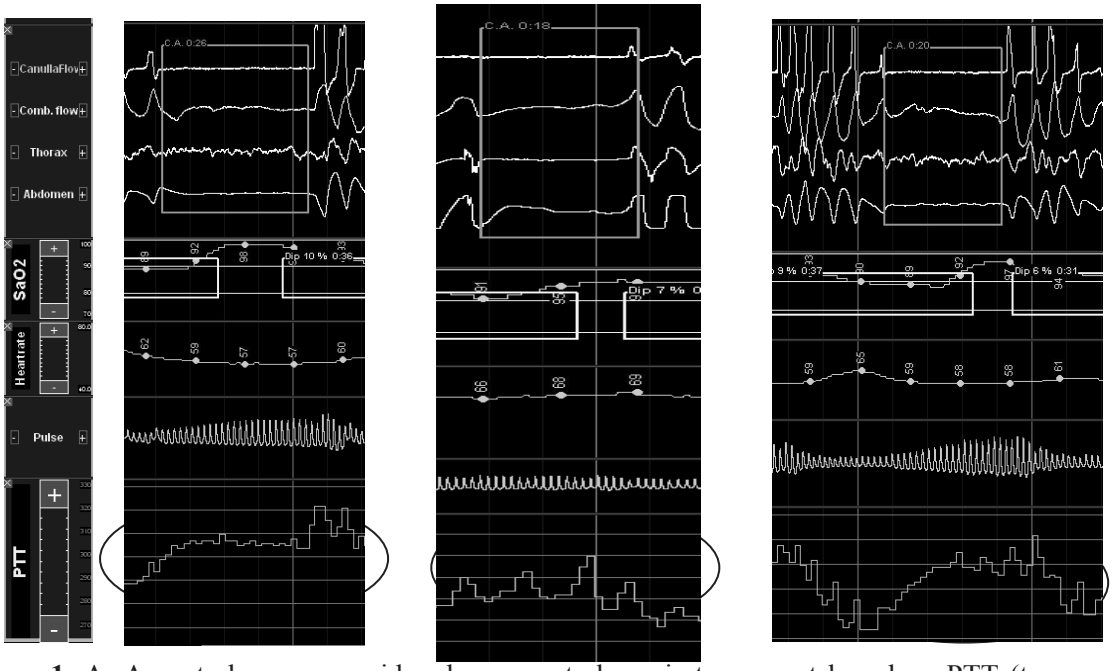

Figure 1. A. A central apnea considered as a central respiratory event based on PTT (true central apnea). B. A central apnea not considered as a central respiratory event based on PTT (false central apnea). C. A central apnea considered as a mixed respiratory event based on PTT.

RESULTS AND DISCUSSION

After the initial scoring procedure (RIP) we analysed 1121 central apneas and could detect 899 (80%) central apneas based on the PTT. After rescoring, based on the combined signals, we could definitely classify 585 (52%) events as true central apneas. After counting the overlooked and undetected central apneas as well, we finaly classified 701 (63%) events as true central apneas (Table 1). All measurements were significantly different compared to each other ($p<0.05$)

Table 1. Patient characteristics and results of the number of central apneas based on respiratory inductance plethysmography (RIP), PTT and combined techniques.

	Baseline (n=12)	*P-value*
Age (years)	56 ±12	
Body mass index (kg/m^2)	29± 5	
Apnea hypopnea index (AHI) (#/h)	43± 17	
FEV1 (% pred)	103 ± 17	
FEV1/VC (% pred)	99 ± 15	
TLC (% pred)	103 ± 7	
PaCO2 (mmHg)	38 ± 3	
PaO2 (mmHg)	86 ± 13	
Central apneas, RIP (#) (%)	1121 (100%)	
Central apneas, PTT (#) (%)	899 (80%)	<0.01
Central apneas, PTT + RIP (#) (%)	585 (52%)	0.01
Central apneas, PTT + RIP + undetected CA (#) (% total)	701(63%)	<0.01

All measurements were significantly different ($p<0.05$).

The gold standard method to measure respiratory effort is by assessment of esophageal pressure swings with an invasive esophageal pressure catheter. It is well known that the use of RIP, a standard method used in clinical routine, overestimates the number of central apneas[2]. Several reasons account for this. First of all, signals obtained from RIP are affected by movement artefacts, particularly in obese patients. In 10% of patients, rib cage and abdominal movement detectors give poor movement signals[5]. Secondly, displacement of the belts throughout the registration could occur and worsen the signal quality.

After combining RIP with PTT to score central apneas, a lower number of central apneas was found, as reported earlier[3]. On the other hand, 27% of the central apneas, based on PTT, were false positive interpretations and 11% of the total definitive true central apneas, were not reliable to interpret or were undetected based on both signals.

PTT is an inferred signal from the pulse signal and the electrocardiogram. Respiratory effort is not the only physiological component inducing fluctuations in PTT. Blood pressure changes, movement artefacts, arousals, displacement of the PPG, all of them reflect in the pulse signal and make it more difficult to interpret and to use PTT as a stand-alone tool to score central apneas.

PTT is also influenced by fluctuations in autonomic tone and by left ventricular isometric contraction time. Hence, pathologicol conditions such as heart failure or use of drugs that modify autonomic tone or cardiac contractility could be confounding factors in analysing variations in PTT. In our study population non of the patients took any important medication. Moreover, changes in compliance of the arterial wall due to aging or atheromatosis are also associated with modified PTT.

Apart from these potential physiological limitations to PTT measurement, misclassifications have been identified of respiratory events induced by artifacts on the PTT signal or occurring in REM sleep[2]. PTT less than 0,2 sec in normotensive subjects usually represents artifacts induced by finger movements. PTT values greater than 0,4 sec are likely artefacts but usually express an authentic decrease in blood pressure when occurring in clusters of five or more spikes and should be marked as artefacts[4].

CONCLUSION

We conclude that assessment of central apneas based on RIP overestimates the number of central apneas. However, when RIP is combined with PTT, central apneas can be detected in

a more reliable way. This prevents overestimation of central apneas and, consequently, an inappropriate treatment choice. Therefore, PTT should be applied routinely in a clinical setting.

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MEASURING SLEEP DISTURBANCES IN ALZHEIMER'S DISEASE

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INTRODUCTION

Sleep becomes more fragmented with age, with increasing nighttime awakening and a greater tendency for daytime sleep. The fragmented sleep-wake pattern which occurs in the elderly becomes even more pronounced in Alzheimer's disease (AD)[1,2]. Several studies show that an increased sleep propensity during the daytime occurs not only in advanced AD patients, but also in patients with mild to moderate AD, and is significantly related to cognitive and functional impairment[3]. These disturbances have a negative impact on quality of life and are one of the primary reasons that caregivers of patients with AD seek institutionalization[4]. For these reasons, sleep disturbances in the AD population are of particular interest.
There are several ways to measure sleep disturbances. Frequently used objective measures are polysomnography and actigraphy. In addition to objective assessment of sleep, subjective perception of sleep is an essential parameter for the diagnosis of sleep disturbances and is often used as a single measure. As perceived sleep duration may differ from objectively measured sleep duration due to cognitive deficits in people with memory complaints, the single use of self-report measures may not always give a realistic quantification of the subject's actual sleep problems.
The aim of the present study, therefore, is to examine whether subjective sleep reports are related to objectively assessed sleep in patients with early stage Alzheimer's disease and healthy age matched controls.

METHODS

31 patients diagnosed with Alzheimer's disease were recruited at the Alzheimer Center of the VU University medical center. The clinical diagnosis of dementia was made according to NINCDS-ADRDA and DSM-IV criteria. None of the patients (11f, 20m, mean age 74.5±3.2) had any interference in their daily living and were all in an early stage of the disease (mean Mini Mental State Exam score (MMSE) of 22±3.8, range 13-29). Also, 14 healthy aged matched controls (4f, 10m, mean age 72.1±4.9 yrs, MMSE ≥28) were included. None of the subjects had other neurological or psychiatric disorders (except mild depression) or was diagnosed with a sleep disorder. The study protocol was approved by the local Ethical Committee and all subjects signed an informed consent.
To measure sleep objectively, subjects wore the Actiwatch (Actiwatch®, CamNtech Ltd, UK) for two weeks on the non-dominant wrist at home. Movement-induced acceleration counts were stored over a 1-min interval. Average recording duration per subject was 12.3±2.6 days and nights. During these two weeks, a pressure pad (RS-components, The Netherlands)

connected to a Hobo U12-06 data logger (LDR, NSL-4962, Silonex, Canada) was placed on the subject's bed underneath their shoulders to determine bed times.
Subjective sleep quality was assessed with the Athens Insomnia Scale (AIS), Pittsburg Sleep Quality Inventory (PSQI) and Sleep Diagnosis List (SDL). A list of all objective and subjective sleep parameters can be found in table 1.
Actigraphy data were analyzed using the Sleep Analysis Software (CamNtech Ltd, UK, version 5.08) with high sensitivity settings. Bed times were visually scored using the Onset Greenline^{TM} software (Onset Computer Corporation, USA, version 1.1.1411.32283).
SPSS version 12.0.1 was used for statistical analyses. Because some variable distributions deviated from normality (Shapiro-Wilk test), Spearman's rho correlations were used to compare the subjective with the objective sleep parameters.
Testing of mean differences between AD patients and controls was performed with independent sample t-test for normally distributed parameters and Mann-Whitney U-test for parameters that were not normally distributed. Significance level was set at $p < 0.05$.
4 AD patients had missing or incomplete actigraphic (<6 nights) and/or pressure pad data. For these patients, only questionnaire data was included for analyses of subjective parameters.

Table 1. List of used objective and subjective sleep parameters

Objective sleep parameters	*Subjective sleep parameters*
Actual sleep time (% and min)	AIS
Actual wake time (% and min)	PSQI
Sleep efficiency (%)	SDL insomnia
Sleep latency (min)	SDL periodic limb movement syndrome (plms)
Sleep bouts (score)	SDL excessive daytime sleepiness (eds)
Wake bouts	SDL narcolepsy
Mean sleep bout time (min)	SDL apnea
Mean wake bout time (min)	SDL psychiatry
Immobile time (% and min)	
Moving time (% and min)	

RESULTS

MMSE ($p< 0.001$), actual wake time ($p< 0.05$), mean wake bout time ($p< 0.05$), immobile time (%) ($p= 0.001$), and moving time (% and min) ($p= 0.001$), differed significantly between AD patients and controls. Except for the MMSE score, AD patients scored higher on these variables. No significant age difference was present between the two groups ($p= 0.06$).
Correlations between subjective sleep parameters showed that, except for the subscales SDL narcolepsy and SDL apnea, highly significant positive correlations were present between the different subjective sleep parameters in AD patients. In the controls, the parameters PSQI, AIS and SDL insomnia showed significant positive correlations ($p< 0.03$).
Both for AD patients and controls, also the greater part of the correlations between objective sleep parameters were highly significant ($p< 0.05$).
Only one significant correlation was found between objective and subjective sleep parameters in AD, i.e. between actual wake time and PSQI ($r= -0.39$, $p= 0.04$). A trend was found for the correlation between sleep latency and SDL plms ($r= -0.36$, $p= 0.06$).
In controls, two significant correlations were found, one between wake bouts and SDL insomnia ($r= -0.55$, $p= 0.04$) and one between sleep latency and SDL plms ($r= -0.53$, $p= 0.05$). Trends ($p= 0.05$ to $p= 0.10$) were found in the parameters PSQI (with actual sleep time

(%), actual wake time (%) and sleep efficiency), SDL insomnia (with sleep efficiency, sleep latency and sleep bouts), SDL narcolepsy (with sleep bouts, wake bouts and mean sleep bout time) and SDL psychiatry (with wake bouts).
Correlations between the MMSE and subjective and objective sleep parameters show trends for actual sleep time ($r= 0.37$, $p= 0.06$), sleep efficiency ($r= 0.33$, $p= 0.09$) and immobile time ($r= 0.35$, $p= 0.08$) in AD patients. In controls, trends were found between MMSE and SDL psychiatry ($r= 0.48$, $p= 0.08$) and between MMSE and mean wake bout time ($r= 0.48$, $p= 0.09$).

DISCUSSION

Summarizing the most important findings of our study, clear differences were found in sleep parameters between patients with AD and controls. Furthermore, poor sleep is related to worse cognition in the AD population. Probably the most important finding is that there is a poor correspondence between objective and subjective measures of sleep, both in AD patients and normal elderly. Hardly any significant correlations between subjective and objective sleep parameters were found, some of which were even in the opposite direction of what was expected. This suggests that there is no good relation between objective and the subjective sleep measurement, and that subjective sleep reports of AD patients, even in an early stage, do not give a realistic quantification of the subject's sleep problems.
To the best of our knowledge, a similar study in AD has not been performed before. There are, however, two studies[5,6] in demented elderly that used caregiver reports instead of sleep questionnaires. Both studies found that questionnaire and actigraphy variables correlated only modestly. Several other studies[7-9] have compared actigraphy to subjective sleep measurements in normal elderly and sleep-disordered patients. Most results are comparable with ours. Some variables correlate only modestly at best and others found nearly a total disagreement between subjective and objective measures of sleep.
This study has several limitations. First of all, the sample size was small, particularly for the group of controls. Secondly, although actigraphy is an accurate non-invasive method to measure sleep objectively, it tends to overestimate sleep compared with polysomnography. Actigraphy cannot distinguish between lying quietly awake and actual asleep[10], which may be especially prevalent in AD. The same arguments hold for the pressure pad. Using the light measurer on both the Actiwatch and the data logger can however reduce this estimation error.
A last possible limitation is the compliance with fulfilment of sleep questionnaires. Controls always filled in the questionnaires themselves but AD patients often received help from their caregiver, and sometimes questionnaires were completely filled in by the caregiver when the patient was no longer capable of doing this him(her)self. This could introduce a bias, even though patients and caregivers were instructed to fill in the patient's opinion, even when this, according to the caregiver, conflicted with the real situation. A possible solution to deal with this problem is to fulfil the questionnaires in the presence of the researcher. An improvement for future studies will be to develop a new subjective sleep questionnaire that is better matched to the sleep parameters of the chosen objective sleep measurement.
Further studies, possibly with larger samples sizes, are necessary to determine the relationships between subjective and objective measures of sleep in AD more precisely. In population based epidemiological studies, data on average sleep duration are typically obtained by subjective measures only. Based on our study and that of others, we recommend, whenever possible, to complement subjective sleep questionnaires with objective measurements.

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Abstracts

LONG SLEEP DURATION IS ASSOCIATED WITH SERUM CHOLESTEROL IN THE ELDERLY: THE ROTTERDAM STUDY

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Introduction. Epidemiological studies have repeatedly found increased mortality associated with both habitual short and long sleep duration. The mechanisms behind these associations are unclear. We investigated whether objectively measured sleep duration, time in bed, and sleep fragmentation were correlated with total cholesterol and high density lipoprotein (HDL) cholesterol in community-dwelling elderly persons.

Methods. This cross-sectional study was conducted among 768 participants of the Rotterdam Study, aged 57 to 97 years. Sleep parameters were assessed with actigraphy, a validated method that infers wakefulness and sleep from arm movement. Cholesterol levels in serum were determined in fasting blood samples. All regression analyses were adjusted for age, gender, body mass index, smoking, depressive symptoms, and heart failure.

Results. Sleep duration was positively correlated with total cholesterol level: $\beta = 0.11$ (95% confidence interval = 0.03 to 0.18) mmol/l per hour of sleep. Persons who slept longer, and spent more time in bed, also had a higher total/HDL cholesterol ratio. A less fragmented sleep was also correlated with higher total cholesterol. Some of these correlations showed significant interactions with age. The correlation between time in bed and total/HDL ratio was mainly driven by persons aged < 65, whereas the relationship between sleep fragmentation and total cholesterol level was most prominent in persons aged ≥ 70.

Conclusion. A longer sleep duration was related to higher total cholesterol level and a higher total/HDL cholesterol ratio. Two separate mechanisms, a longer time in bed and sleep fragmentation, seem to explain these correlations in different age categories.

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Van den Berg JF, Miedema HME, Tulen JHM, Knuistingh Neven A, Hofman A, Witteman JCM, Tiemeier H. Long sleep duration is associated with serum cholesterol in the elderly: the Rotterdam Study. Psychosomatic Medicine 2008 Nov;70(9):1005-11

EFFECT OF SLEEP DEPRIVATION ON SLEEP AND THE SLEEP EEG IN R192Q $CA_V2.1$ MIGRAINE MICE

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Introduction. Mutation R192Q in $Ca_v2.1$ channels causes Familial Hemiplegic Migraine type 1. In a knock-in mouse model this mutation results in increased calcium influx and neurotransmitter release. $Ca_v2.1$ channels are common targets of G-protein linked neuromodulation. Therefore, mutation R192Q causes reduced susceptibility to G-protein inhibition. Adenosine and GABA act on G-protein coupled receptors and are both known to be involved in sleep regulation. A pilot study showed that R192Q mice sleep less than wildtype (WT) mice.

Methods. To investigate the influence of the channel mutation on sleep regulation, WT and R192Q mice were kept in 12:12 h LD cycles and EEG and EMG electrodes were implanted. After recovery and adaptation, a 24-h baseline day (both genotypes n=7) a 6-h SD and 18-h recovery (R192Q n=4, WT n=5) were recorded. Vigilance states were determined and EEG spectral analysis was performed.

Results. Over 24 h R192Q mice were 10% more awake than WT mice ($p<0.01$ t-test). This was attributable to a general reduction in NREM sleep. Waking episodes were longer and NREM sleep episodes were shorter in R192Q mice. REM sleep was not affected. The baseline time course of slow-wave activity (SWA, EEG power density between 0.75-4.0 Hz) did not differ between the genotypes. After SD both genotypes displayed an initial increase in SWA ($p<0.05$, paired t-test). This increase in SWA was significantly higher in WT compared to R192Q ($p<0.05$, t-test) and gradually declined during recovery in both genotypes.

Conclusion. R192Q mice show less NREM sleep during baseline and an attenuated increase in SWA after SD. These results are similar to those found in human short-sleepers who also display a smaller increase in SWA after SD. The data suggest that R192Q mice have a short sleeper phenotype.

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THE EFFECT OF REPEATED SHORT SLEEP DEPRIVATIONS ON SLEEP AND THE SLEEP EEG

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Introduction. Sleep regulation is realized by homeostatic and circadian processes. The homeostatic component is reflected by slow-wave activity (SWA) in the NREM sleep EEG, while high frequency activity (>10 Hz, HFA) is thought to be influenced by the circadian factors. We investigated whether daily changes in sleep and HFA are independent of changes in SWA.

Methods. Rats (n=4) were implanted with EEG and EMG electrodes and kept in 12h:12h light/dark cycles for the recovery period. Subsequently the animals adapted to constant dark conditions (DD) for at least 1 week. A baseline (BL) day was recorded followed by "short-day protocol" - 2h sleep deprivation followed by 2 h rest for 2 days. The vigilance states were determined and EEG spectral analysis between 0.1-25.0 Hz was performed.

Results. The amount of sleep over 24 h of the "short-day protocol" was less (43.3±1.7% SE) compared to baseline (51.5 ±1.2%, $p<0.05$, t-test). Circadian changes in vigilance states was reduced to 25 % of baseline ($p<0.05$, t-test). SWA (1.1-4.0 Hz) in NREM sleep did not show a significant circadian modulation ($p>0.5$, ANOVA 4-h intervals) during the protocol, while the power density in spindle range (11.1-15.0 Hz) and frequencies between 15.1-25.0 Hz showed strong circadian modulation ($p<0.05$, ANOVA 4-h intervals) which did not differ from baseline ($p>0.5$, ANOVA). Analysis of the time course of SWA and power density in the spindle range in the first 7 min of a NREM sleep episode confirmed these results, with no circadian modulation in SWA but circadian changes in the spindle range and a time course within the NREM sleep episode identical to baseline.

Conclusions. The present data show that, in a contrast to SWA, HFA is not influenced by sleep homeostatic mechanisms and displays significant circadian modulation with endogenous origin.

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SLEEP AND SLEEP HOMEOSTASIS IN CONSTANT DARKNESS IN THE RAT

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Introduction. According to the two-process model of sleep regulation, a homeostatic Process S increases during waking and decreases during sleep. The time course of Process S can be derived on the basis of changes in vigilance states and changes in electroencephalogram slow-wave activity (SWA, activity below 4 Hz) during non-rapid eye movement (NREM) sleep. In most mouse strains an optimal fit between S and SWA was achieved with one increasing (active during waking and REM sleep) and one decreasing time constant (active during NREM sleep) for Process S. However, in the rat, systematic deviations in the light and dark period were observed, which were resolved by introducing different decreasing time constants between the light and dark period.

Methods and results. The present study shows that this difference between rest (light) and active (dark) phase remains, and may even be larger, after animals are adapted to constant dark conditions for at least a week. In addition, the data show that the build-up rate of SWA at the onset of a NREM sleep episode is slow compared to the increase rate under light-dark conditions, and that this build-up rate changes with circadian phase. The slow build-up rate introduces a systematic error between the simulation of Process S (Figure, gray area) and SWA in NREM sleep (white area).

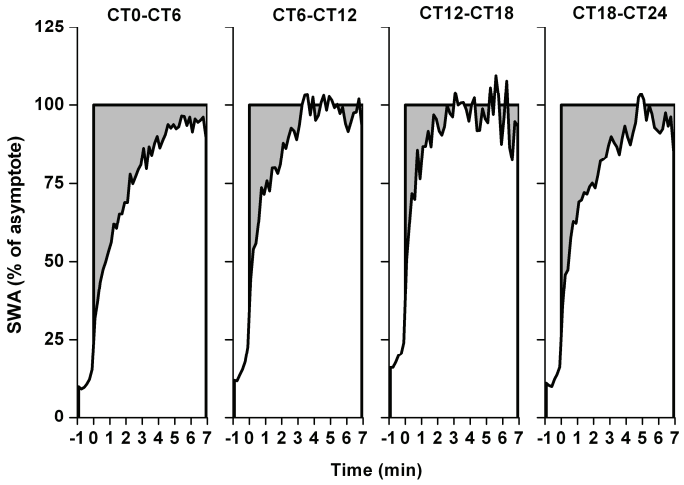

Figure. The time course of SWA within NREM sleep episodes during a baseline day. The gray area behind SWA represents the hypothetical process S.

Conclusion. The circadian modulation of the build-up rate may, together with circadian changes in NREM sleep episode duration, be the source of the necessity of introducing a difference in the decreasing time constant between the rest and active phase.

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HOMEOSTATIC AND CIRCADIAN PROCESSES CONTRIBUTE JOINTLY TO THE MAGNITUDE OF SYSTEMATIC INDIVIDUAL DIFFERENCES IN PERFORMANCE IMPAIRMENT DURING SLEEP DEPRIVATION

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Introduction. Studies of trait individual differences in cognitive impairment during sleep deprivation have focused primarily on performance averaged over the circadian cycle, leaving homeostatic and circadian processes intertwined. Here we use mathematical modeling to 1) separate systematic individual differences from noise and 2) examine the contributions of the two processes to the magnitude of individual differences in vulnerability to sleep deprivation.

Methods. As part of a larger laboratory study, 12 healthy young adults (aged 22-37y; 5 females) were sleep-deprived for 62h following two baseline nights (10h TIB each). Performance on a 10-minute psychomotor vigilance test (PVT) was assessed at 2h intervals through most of the sleep deprivation period. Using a standard two stage approach for quantifying individual differences, each subject's time series of PVT lapses (RT>500ms) was fitted with the two-process model of sleep regulation using a linear combination of the homeostatic and circadian equations. The mean and standard deviation over subjects of modeled performance were computed for each measurement time. The standard deviation served as an index of the magnitude of systematic individual differences for each time point.

Results. Across time points, the correlation of the magnitude of individual differences with mean modeled homeostatic pressure was 0.92, while the correlation with mean modeled circadian rhythm was 0.26. The correlation with mean modeled performance was 0.99, exceeding the correlation with either homeostatic pressure or circadian rhythm alone. The correlation of individual differences with mean performance over subjects was still high (0.934) when using mean raw instead of modeled performance, indicating that the modeling results represented the data adequately.

Conclusion. Although individual differences in circadian and homeostatic processes may contribute independently to individual differences in performance impairment during sleep deprivation, the findings suggest that the magnitude of individual differences is predominantly determined by a single performance-degrading force in which the homeostatic and circadian processes are combined.

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MODELING RECOVERY AFTER CHRONIC SLEEP RESTRICTION: SLEEP EXTENSION CAN PROVIDE RECUPERATION OF PERFORMANCE BUT MAY BE NEITHER NECESSARY NOR SUFFICIENT

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Introduction. Little is known about effective strategies for recuperation from performance impairment due to chronic sleep loss. Mathematical modeling may be used to extrapolate from laboratory data sets to generate targeted hypotheses for experimental investigation of this issue. We consider a recently developed mathematical model of sleep/wake homeostatic effects on cognitive performance that may generate new insights into recuperation following cognitive performance degradation due to chronic sleep restriction.

Methods. We used an extension of the two-process model described in a companion abstract (McCauley and colleagues). The model was calibrated using PVT lapse data from a laboratory study involving 14 days of sleep restriction to 8h, 6h or 4h TIB daily, or 3 days of total sleep deprivation (N=48; 72.4% of variance explained). The model was validated using PVT lapse data from a laboratory study involving 7 days of sleep extension/restriction to 9h, 7h, 5h or 3h TIB per day (N=56; 72.2% of variance explained).

Results. Several predictions were made based on an analysis of the dynamics of the model. First, for chronic sleep restriction to no less than ~4h TIB per day, performance converges to a steady state of impairment over days. Second, from the steady state, performance deficits diminish when daily TIB is increased even if still restricted. Third, if increased TIB occurs only intermittently, then performance deficits subsequently converge back rapidly to a steady state of impairment.

Conclusion. Based on mathematical model predictions that extrapolate from observations (>100 subjects) in chronic sleep restriction experiments, we derive the following hypotheses: 1) It is possible to recuperate from performance impairment after chronic sleep restriction by reverting to a schedule of sustained non-restriction, without requirement for further sleep extension (or making up for "lost" sleep); and 2) Sleep extension ("oversleeping") may accelerate recuperation but is not sufficient to restore performance unless followed by a schedule of sustained non-restriction. Experimental confirmation of these hypotheses will have significant implications for scheduling in operational settings.

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COGNITIVE PERFORMANCE PREDICTIONS FROM A NEW BIOMATHEMATICAL MODEL OF SLEEP/WAKE HOMEOSTASIS

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Introduction. Cognitive performance has been mathematically modeled on the basis of two biological processes: sleep/wake homeostasis and circadian rhythmicity. We showed that published equations for the homeostatic process generalize to a broader class of models formulated as coupled nonhomogeneous first-order ordinary differential equations (McCauley et al., J. Theor. Biol., 2008). We investigated the dynamic properties of this model class.

Methods. Model parameters were estimated using psychomotor vigilance test (PVT) lapse data from a laboratory experiment involving 14 days of sleep restriction (4h, 6h, or 8h TIB/day), or 3 days of total sleep deprivation (N=48). From the generalized model equations, coupled difference equations were derived to predict performance at the onset and end of successive wake periods. Mathematical analysis of these equations revealed that a bifurcation (i.e., qualitative change in model behavior) could occur when a specific amount of daily wakefulness is exceeded.

Results. For the estimated parameter values, the bifurcation was predicted to occur at 20.2h wakefulness (3.8h sleep) per day. For daily wakefulness <20.2h, the model converged to a stable state of (impaired) performance. For daily wakefulness >20.2h, no stable state was achieved, and impairment was predicted to escalate over days. These predictions were confirmed by another laboratory experiment, involving 7 days of sleep restriction (3h, 5h, 7h, or 9h TIB/day; N=56). The model explained 72.2% of the variance, and a disproportionate accumulation of performance deficits was observed in the 3h TIB condition.

Conclusion . Analysis and validation of our generalized model of sleep/wake homeostatic effects on cognitive performance revealed that sleep restriction to below a threshold estimated at 3.8h per day results in a fundamentally different (disproportionately accelerated) build-up of PVT performance impairment across days. Since much slow wave sleep (SWS) normally occurs within the first ~3.8h of nocturnal sleep, this suggests that chronic sleep restriction causes cumulative deficits considerably faster when SWS is curtailed.

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NO SIGNIFICANT EFFECT OF SLEEP DEPRIVATION ON IMPULSIVITY IN A DELAY DISCOUNTING TASK

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Introduction. Executive functioning is thought to be especially vulnerable to impairment due to sleep loss. In previous studies, however, we found that some aspects of executive functioning are resilient to total sleep deprivation (TSD). These studies focused on "cold" cognitive tasks, devoid of affective cognition. In the present study, we consider a delay discounting (DD) task, which requires motivational reasoning and affective or "hot" cognition. In each of 27 trials, subjects choose between receiving two different amounts of imaginary money—one amount is smaller but rewarded immediately, while the other is larger but rewarded after varying time delay. Choices based on reward magnitude and delay are used to estimate how value declines subjectively with delay. This is described with a hyperbola with rate parameter k, which reflects impulsivity. We use the DD task here to examine the effects of TSD on impulsivity in decision making.

Methods. 23 healthy adults (22-38y; 11f) spent 7 consecutive 24h days in a laboratory. 12 subjects were randomized to 62h TSD preceded and followed by 2 days with 10h TIB; 11 controls had 10h TIB each night. The DD task was administered during baseline, at 51h TSD (or control), and after 2 days of recovery, with circadian timing held constant. Different but equivalent versions of the task were presented in randomized order.

Results. Mixed-effects ANOVA showed no effect on k values of test session ($F=1.32$, $P=0.28$), condition ($F=2.05$, $P=0.16$), and condition by session interaction ($F=0.09$, $P=0.92$). The average TSD effect on k values was -0.002±0.006—a non-significant decrease in impulsivity. There was a significant effect on response time (RT) of test session ($F=17.7$, $P<0.001$), with subjects responding progressively faster (practice effect), while there was no effect of condition ($F=1.31$, $P=0.26$) or interaction ($F=2.44$, $P=0.10$). The average TSD effect on RT was an increase of 605±230ms (planned contrast: $t=-2.16$, $P=0.04$).

Conclusion. The effect of TSD on k values was non-significant and negligible compared to effects in studies involving increased working memory load, which documented large increases in k. The present results indicate that under controlled laboratory circumstances, 51h TSD did not alter impulsivity, providing preliminary evidence that some aspects of "hot" executive functioning may be resilient to sleep loss. This finding needs to be interpreted in the context of subjects responding slower on the DD task during TSD.

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GENERAL INTELLECTUAL FUNCTIONING DOES NOT PREDICT PERFORMANCE IMPAIRMENT ON THE PSYCHOMOTOR VIGILANCE TEST DURING TOTAL SLEEP DEPRIVATION

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Introduction. There are substantial, trait-like individual differences in performance impairment on the psychomotor vigilance test (PVT) during total sleep deprivation (TSD). Based on theories of cognitive reserve and compensatory brain mechanisms, we hypothesized that overall cognitive ability might predict individual differences in PVT impairment due to TSD. In the present study, we aimed to test this hypothesis using the Shipley Institute of Living Scale (SILS), a validated measure of general intellectual functioning.

Methods. As part of a larger study, 12 healthy young adults (age 27.4±4.5; years of education 14.3 ± 1.9; 5 females) spent 7 consecutive days in a laboratory. Following two baseline days with 10h time in bed, subjects underwent 62h of TSD. Performance on a 10min PVT was tested at 2h intervals throughout most scheduled wakefulness. PVT number of lapses (RT>500ms) was averaged across a baseline period from 0h to 14h awake; across the 24h TSD period from 14h to 38h awake, and across the subsequent 24h TSD period from 38h to 62h awake. The SILS was administered during baseline. Raw SILS scores were converted to Wechsler Adult Intelligence Scale (WAIS) estimates of IQ stratified by age using established benchmarks.

Results. SILS scores ranged from 51 to 69, corresponding to IQ estimates ranging from 103 to 119. PVT performance impairment showed considerable individual differences similar in magnitude to that reported in earlier studies. No significant relationship was found between the Shipley scores and average PVT lapses during 14h-38h awake (r= -0.17, P=0.59); during 38h-62h awake (r=0.07, P=0.82); or during the two intervals of total sleep deprivation combined (r=-0.05, P=0.88). Results were similar for estimated IQ scores: there was no significant relationship with average PVT lapses during 14h-38h awake (r=–0.13, P=0.68); during 38h-62h awake (r=0.09, P=0.79); or during these intervals of total sleep deprivation combined (r=–0.02, P=0.95). Correcting for baseline PVT performance did not substantively alter these results.

Conclusions. In our sample of healthy young adults with average to above-average estimated IQ, individual differences in PVT performance impairment during total sleep deprivation were not predicted by SILS scores, or by IQ scores corrected for age. In this population, vulnerability to PVT performance impairment due to TSD does not appear to be a function of general intellectual functioning.

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CHANGES IN AFFECT SCORES ACROSS 62 HOURS OF TOTAL SLEEP DEPRIVATION

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Introduction. Numerous studies have demonstrated that sleep deprivation has considerable adverse effects on cognitive performance, but the effects on affect have not been systematically investigated. During extended wakefulness, effects of time awake (sleep homeostasis) interact with time of day (circadian rhythm) in degrading cognitive functioning. Previous studies documented reductions in positive affect with time awake and circadian rhythm, but yielded mixed results for negative affect. We investigated positive and negative affect during 62h of total sleep deprivation, relative to a control condition, with repeated measures across all hours of the day under strict laboratory control.

Methods. N=23 healthy adults (22-40y; 11 females) spent 7 consecutive 24h days in the laboratory. 12 subjects were randomized to 62h of total sleep deprivation, which was preceded and followed by two 10h nocturnal sleep periods. 11 controls were allowed to sleep 10h each night. Every 2h throughout most of the scheduled waking periods, the Karolinska Sleepiness Scale (KSS; a 9-item Likert-type instrument assessing subjective sleepiness) and the Positive and Negative Affect Schedule (PANAS) were administered. KSS and PANAS scores measured during the 62h sleep deprivation period were subjected to mixed-effects ANOVA to examine temporal changes during sleep deprivation. Furthermore, PANAS and KSS scores obtained across the whole study were analyzed using mixed-effects ANOVA to compare between the sleep deprivation and control conditions.

Results. During sleep deprivation, there were effects of time for KSS subjective sleepiness ($F=13.4$; $P<0.001$) and for PANAS positive affect ($F=8.9$; $P<0.001$) and negative affect ($F=3.0$; $P<0.001$). Subjective sleepiness and negative affect increased across time awake, with a circadian rhythm superimposed thereon so that the strongest effects occurred in the early morning (~07:00). Over the sleep deprivation period, relative to baseline, negative affect increased by ~10% of the scale range. Positive affect exhibited the same profile but inversed, with the average magnitude of changes exceeding 25% of the scale range. KSS scores were predictive of affect scores, with likelihood ratio tests revealing that models with subjective sleepiness as a covariate described the data more adequately than models without this covariate, for both positive affect ($\chi^2=17.1$, $P<0.001$) and negative affect ($\chi^2=21.4$, $P<0.001$). Comparison between the sleep deprivation and control conditions across all the measurements that these conditions had in common exposed significant interactions of condition by time for subjective sleepiness ($F=7.0$, $P<0.001$), positive affect ($F=2.4$, $P<0.001$) and negative affect ($F=1.7$, $P=0.012$). The experimental condition diverged from the control condition during the sleep deprivation period, but returned to control levels after recovery sleep.

Conclusion. Subjects showed a progressive deterioration of affect across 62h of total sleep deprivation, modulated by a pronounced circadian rhythm. These systematic changes in both

positive and negative affect occurred without experimental perturbation of the affective system other than by extended wakefulness. The temporal profile of changes was similar to that seen in subjective sleepiness, suggesting a common underlying mechanism. The finding that PANAS scores varied with sleep loss and circadian rhythm suggests that clinical assessments of affective functioning should account for sleep/wake and circadian states when comparing against normative data.

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INDEPENDENT DIMENSIONS OF TRAIT INDIVIDUAL DIFFERENCES IN SLEEP ARCHITECTURE

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Introduction. In a recent study of trait individual differences in sleep architecture, PSG-derived sleep variables clustered in three dimensions tentatively interpreted as representing sleep duration, sleep intensity, and sleep discontinuity. Here we investigate the replicability of this finding in a different study of trait individual differences.

Methods. As part of a larger study, 21 healthy subjects (ages 21-38y; 9 women) underwent two 36h total sleep deprivation sessions during separate, strictly controlled laboratory visits. Each sleep deprivation session was preceded by baseline sleep and followed by recovery sleep (12h TIB, 22:00-10:00) in the laboratory. PSG records of the two baseline and the two recovery sleep periods were visually scored by R&K criteria. Standard sleep parameters were extracted, and slow-wave activity (SWA; 0.75-4.5Hz) was computed from the NREM sleep EEG for four derivations (Fz,C3,C4,Oz vs. A1/A2). A total of 13 sleep records was discarded because of equipment failure. For each of the remaining 71 records, 18 sleep variables were entered into principal components analysis (PCA) to determine which variables covaried over subjects and across nights. Sleep variables with absolute factor loadings >0.5 were used for interpretation.

Results. Three factors were retained in the PCA following inspection of the scree plot. The following independent dimensions of sleep architecture emerged after varimax rotation: 1) sleep duration (TST, sleep efficiency, S2, REM, number of sleep cycles, and negative loadings for REM latency and WASO); 2) sleep intensity (SWS, and SWA for the four derivations); and 3) sleep discontinuity (S1, movement time, and stage transitions). Latencies to S1, S2 and SWS did not load >0.5 on these dimensions.

Conclusions. This study yielded converging evidence that there are at least three independent components of systematic individual variability in sleep architecture. The results are congruent with our earlier finding that individual differences in nocturnal PSG-derived sleep variables cluster in three dimensions, which appear to represent sleep duration, sleep intensity, and sleep discontinuity.

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INSULIN SENSITIVITY AND PANCREATIC BETA-CELL FUNCTION IN NARCOLEPSY

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Introduction. Besides excessive daytime sleepiness, patients with narcolepsy often have an increased body mass index (BMI) and disturbed nocturnal sleep. The latter are both risk factors for the development of type II diabetes mellitus (T2DM). Although a higher prevalence of T2DM has been reported in narcolepsy patients. The underlying mechanisms are unknown. We investigated whether narcolepsy patients have altered insulin sensitivity and pancreatic beta-cell function, making them more susceptible to develop T2DM.

Methods. We studied 8 medication-free, hypocretin deficient male narcolepsy, and 8 healthy, age, sex and BMI matched controls. Blood samples were taken at 10-min. intervals from 2 hours before to 2 hours after standardized meals (dinner and breakfast). Insulin sensitivity was estimated by calculating the area under the curve (AUC) of the plasma glucose/insulin ratios. In addition, the homeostatic model assessment (HOMA) was used to determine beta-cell function and insulin resistance as percentages of a normal reference population.

Results. The mean AUC of the glucose/insulin ratios around dinner was lower in narcolepsy patients than in controls (60.9 ± 9.3 vs. 93.9 ± 11.9, p = 0.047), but this effect diminished when data were pooled over the two distinct time-frames (71.3 ± 10.4 vs. 108.1 ± 15.6, p = 0.071). Using HOMA, narcolepsy patients also appeared more resistant, although the difference with controls did not reach statistical significance (1.7 ± 0.3 vs. 1.1 ± 0.2, p= 0.075) The percentage of beta-cell function was higher in narcolepsy patients (115.6 ± 12.8 vs. 78.9 ± 8.3, p = 0.031), probably reflecting a compensatory increase of beta-cell capacity.

Conclusion. These data strongly suggest, but do not definitely prove, that patients with narcolepsy are insulin resistant as compared to controls matched for age, sex and BMI, which can explain their propensity to develop type 2 diabetes mellitus.

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THE VALIDITY OF ACTIGRAPHY IN CHILDREN WITH ADHD AND INSOMNIA: A COMPARATIVE STUDY WITH POLYSOMNOGRAPHY

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Introduction. To examine the accuracy of actigraphy measurements in children with Attention-Deficit/Hyperactivity Disorder (ADHD) and chronic sleep onset insomnia by analyzing agreement between actigraphy and polysomnography (PSG) on several sleep parameters using Bland and Altman's limits of agreement.

Methods. 13 Unmedicated children with ADHD and insomnia, aged 6-11 years, were included. Sleep onset, wake up time, total sleep time and actual nocturnal awake time were monitored with actigraphy (automatically scored, with or without manual verification) and PSG for one single night. Parents completed sleep logs to report on sleep onset and wake up time.

Results. For sleep onset and wake up time, only manually verified actigraphy measurements fell between the pre-defined acceptable range of ±20 minutes (-13.7 to 17.4 and -17.5 to 26.6 minutes for sleep onset and wake up time respectively). Actual awake time was consistently overestimated by actigraphy (automatically scored) as compared to PSG (mean difference: 66.2 minutes) and showed large limits of agreement (-137.95 to 5.64 minutes).

Conclusion. In children with ADHD and insomnia, agreement between actigraphy and PSG was higher for sleep onset than for wake up time and total sleep time measurements. Actigraphy provided more reliable sleep data when automatically scoring was followed by manual verification. However, careful interpretation is necessary when actigraphy is used to measure actual awake time in children with ADHD and insomnia.

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EUROPEAN DATA FORMAT – RECENT DEVELOPMENTS

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The European Data Format (EDF) and its compatible super-specification (EDF+) are a standard for storage, archival and exchange of EEG and PSG recordings and their scorings (www.edfplus.info). They were published in 1992 and 2003, respectively. In this period, EDF became the de-facto standard in most European multi-center PSG studies and many sleep labs. This abstract reports the developments since then.

More than 80 manufacturers now support EDF(+). Many of them make PSG systems, but the majority now also applies EDF(+) to EEG, ECG, EMG or general biosignal recordings. After the Philadelphia meeting in 2006 (http://physionet.org/npsg-standards), during which scientists met the industry, about twenty American companies turned to EDF(+). New implementations are usually based on EDF+ rather than EDF. Most multi-center PSG studies in the USA are now based on EDF(+).

Last year, one of the working groups of the European Normalization Committee (CEN) has started the development of a formal standard. This standard will be fully compatible to EDF+ but also contain a specification for video recordings. The internal on-line video format will be left free, but an off-line Theora exporter may be part of the specification. Theora is an open video format, not limited by patents. Independent of the applied format, the video file will be time-synchronized to the EDF+ file by noting its start-date/time in the filename, like in: NL_012348168_21-APR-2007_02h45m49.102s.ogv. The first part, in this case NL_012348168, must match the first part of the EDF+ filename and identifies the patient.

The EDF+ specs were finalized in 2003, but allow newly defined standard labels and annotations to be published on the EDF+ website. This flexibility was exploited shortly after the new AASM criteria for manual sleep scoring became available in 2007. The EDF+ standard annotations now also contain the AASM-defined sleep stages, respiratory and cardiac events, and so on. The EDF+ standard labels contain ‘EMG RAT’ and ‘EMG LAT’.

Besides many commercial packages, several free programs became available. These have different useful features but particularly can check EDF(+) compatibility of commercial recordings as well as their contents. Several researchers have made EDF(+) recordings available for download. Links to software and data are on www.edfplus.info.

EDF+ is widely used in Europe and the USA as the standard format for EEG, PSG and increasingly also for ECG and EMG. It now also supports AASM scorings and video. Software and recordings are freely available. Data can now be exchanged between equipment and software from most manufacturers.

IMPAIRMENT OF ENDOGENOUS MELATONIN RHYTHM IS RELATED TO THE DEGREE OF CHRONIC KIDNEY DISEASE (CREAM STUDY)

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Introduction. Chronic Kidney Disease (CKD) is associated with an increased prevalence of sleep disturbances, which have a major influence on quality of life and morbidity. Melatonin, which is normally only secreted at night, plays a major role in the circadian sleep-wake rhythm regulation. Recently, we have found that the nocturnal endogenous melatonin rise, which is associated with the onset of nocturnal sleep propensity, is absent in hemodialysis patients. Information on melatonin rhythm in patients before initiating dialysis treatment is limited. Second, clear relationships exist between melatonin, core body temperature and cortisol in healthy subjects. In CKD, no data are available on these relationships. The aim of this study was to characterize the rhythms of melatonin, cortisol and temperature in relationship to renal function in patients with CKD.

Methods. Over a 24-hour period, blood samples of 28 subjects with various degrees of renal function were collected. Blood samples were collected every 2 hours and stored at -70°C until assay. An intestinal telemetric sensor was used to measure temperature. Among the exclusion criteria were acute renal failure, use of hypnotics and use of exogenous melatonin and erythropoietin. The existence of a circadian rhythm was examined by fitting of a cosine function to the melatonin, cortisol and temperature time series. Furthermore, Pearson correlation analysis was performed. Renal function was expressed as Cockcroft Gault Glomerular Filtration Rate (GFR).

Results. The mean age was 71 years (SD: 6.8). In the study 8 female patients were included. The mean GFR was 61 (SD: 34.9). The subjects exhibited melatonin (n= 24), and cortisol (n= 22) rhythms. GFR was significantly correlated to melatonin amplitude ($r = 0.59$, $p = 0.003$) and to total melatonin production ($r = 0.51$, $p = 0.01$), but not to temperature or cortisol. Interestingly, no associations were found among the parameters of temperature, melatonin and cortisol.

Conclusion. The data warrant follow-up research into circadian rhythms in patients with CKD. As melatonin levels decreased with advancing renal dysfunction, exogenous melatonin may be evaluated in the management of sleep disturbances in patients with CKD.

EARLY INDICATIONS OF DELIRIUM BY QUANTIFICATION OF 24-HOUR MOTOR ACTIVITY PATTERNS

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Introduction. Delirium after cardiac surgery is a risk factor for adverse outcome and even death. Disturbance of motor activity is a core feature of delirium, but hypoactive delirium often remains unrecognized. Objective quantitative measurements of motor activity patterns might improve early diagnosis. We explored wrist-actigraphy as a tool to objectively quantify postoperative recovery of 24-hour rest-activity patterns to improve the early recognition of delirium after surgery.

Methods. Motor activity was recorded by wrist-actigraphy after cardiac surgery in 88 patients over 65 years of age. Patients were assessed daily by using the CAM-ICU. Our final analyses were based on 32 non-delirious patients and 38 patients who were delirious on the first day after surgery.

Results. The delirious patients showed lower mean activity levels during the first post-operative night ($p<.05$), and reduced restlessness during the first day ($p<.05$), compared to non-delirious patients (table 1). Also, the mean activity of the 5 hrs with lowest activity within the first 24 hrs in the delirious patients was lower during the first day as compared to the non-delirious patients ($p=.01$).

Conclusions. Already at a very early stage after cardiac surgery, a difference in motor activity was observed between patients with and without a delirium. As an unobtrusive method, actigraphy has the potential to be a screening method that leads to early diagnosis and treatment of delirium.

Table 1: Median (minimum, maximum) values of the motor activity parameters during the first post-operative night and day.

	Non-delirious	*Delirious*	*Z-, p-value*
Immobility			
Night 1	371 (222, 420)	395 (22, 420)	*-1.61; ns*
Day 1	632 (239, 863)	732 (50, 925)	*-1.70; 0.09*
Mean Activity			
Night 1	5 (0, 47)	2 (0, 80)	*-1.97; 0.05*
Day 1	32 (9, 92)	25 (3, 200)	*-1.77; 0.08*
Restlessness			
Night 1	25 (0, 82)	21 (0, 162)	*-1.07; ns*
Day 1	73 (33, 130)	53 (11, 125)	*-2.13; 0.03*
L5	172 (0, 2431)	14 (0, 2523)	*-2.53; 0.01*
M10	2065 (549, 6969)	1712 (226, 5498)	*-1.24; ns*

L5: mean activity of the 5 hrs with the lowest activity within the first 24 hrs. M10: mean activity of the 10 hrs with the highest activity within first 24 hrs.

Based on: Osse RJ, Tulen JHM, Hengeveld MW, Bogers AJJC. Screening methods for delirium: early diagnosis by means of objective quantification of motor activity patterns using wrist-actigraphy. Interactive CardioVascular and Thoracic Surgery, 8: 344-348, 2009.

SLEEP DISTURBANCES IN MYOTONIC DYSTROPHY TYPE 2: A COMPARISON WITH MYOTONIC DYSTROPHY TYPE 1 AND HEALTHY CONTROLS

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Introduction. Excessive daytime sleepiness (EDS) is the most frequent non-muscular symptom of myotonic dystrophy type 1 (DM1), a dominantly inherited neuromuscular disease characterized by muscle weakness, myotonia, and multisystem involvement. Myotonic dystrophy type 2 (DM2) has similar clinical characteristics as DM1, but there have been no studies into the prevalence of EDS, nocturnal sleep disturbances, or fatigue. We performed a systematic analysis into these symptoms in a cohort of DM2 patients, and compared these to a matched group of adult-onset DM1 patients and to healthy controls.

Methods. Twenty-nine genetically proven DM2 patients completed the Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality Index (PSQI) and the Checklist Individual Strength (CIS). The results were compared to those of 29 adult-onset DM1 patients and 65 healthy controls.

Results. Almost half (45%) of DM1 patients had EDS (ESS>10) compared to only 6.9% of DM2 patients and 6.2% of healthy controls. The mean ESS score differed significantly as well (DM1: 9.6, DM2: 4.3, controls: 5.4, $p < 0.000$). Comparable to the DM1 group, patients with DM2 experienced severe fatigue (mean CIS-fatigue DM2: 38.7 ± 13.1, DM1: 42.9 ± 8.5, versus healthy controls 21.1 ± 11.1, $p=0.00$). Overall sleep quality was poor (PSQI > 5) in both the DM2 and DM1 group (PSQI DM2: 6.5 ± 3.0; DM1 6.2 ± 3.7) in contrast to healthy controls (4.3 ± 3.0, $p=0.003$). The PSQI subscore *sleep disturbances* was significantly higher in the DM2 group than in the DM1 group and healthy controls. (DM2: 1.4 ± 0.8, DM1 1.0 ± 0.7, healthy controls 1.1 ± 0.5, $p=0.039$ and $p=0.008$ respectively). This difference was mainly due to sleep-disrupting pain in DM2 patients. The subscores *sleep duration, sleep efficiency* and *the use of sleeping medication* did not differ significantly between the three groups.

Conclusion. In contrast to DM1, EDS is not part of the clinical picture of DM2. However, DM2 patients show high levels of experienced fatigue and poor sleep quality, comparable to those of DM1 patients. Sleep in DM2 is more often disturbed compared to DM1, mainly because of nocturnal pain.

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HYPNAGOGIC HALLUCINATIONS AND "PSYCHOTIC" SYMPTOMS IN NARCOLEPSY: A COMPARISON WITH CONTROL SUBJECTS AND SCHIZOPHRENIC PATIENTS

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Introduction. Patients with narcolepsy often experience pervasive hypnagogic hallucinations, sometimes even leading to confusion with schizophrenia. We aimed to provide a detailed qualitative description of hypnagogic hallucinations and other "psychotic" symptoms in patients with narcolepsy and contrast these with schizophrenia patients and healthy controls. We also compared the prevalence of formal psychotic disorders between narcolepsy patients and controls.

Methods. We used SCAN 2.1 interviews to compare "psychotic" symptoms between 60 patients with narcolepsy, 102 with schizophrenia, and 120 matched population controls. In addition, qualitative data was collected to enable a detailed description of hypnagogic hallucinations in narcolepsy.

Results. There were clear differences in the pattern of hallucinatory experiences in narcolepsy versus schizophrenia patients. Narcoleptics reported multisensory "holistic" hallucinations rather than the predominantly verbal- auditory sensory mode of schizophrenia patients. Other psychotic symptoms such as delusions were not more frequent in narcolepsy compared to population controls. In addition, the prevalence of formal psychotic disorders was not increased in patients with narcolepsy. Almost half of narcoleptics reported moderate interference with functioning due to hallucinations, mostly due to related anxiety.

Conclusions. Although hypnagogic hallucinations in narcolepsy are frequent and pervasive, we did not find a higher frequency of psychotic disorders compared to population controls, which points to a relatively healthy insight. Hypnagogic hallucinations are different on a phenomenological level (form and type) from hallucinations of schizophrenic patients.

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LACK OF SLEEP IN MANAGERS: STATE OF THE WORLD ECONOMY TO BLAME?

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* Data survey commissioned by Royal Philips Electronics, conducted by TNS

Introduction. Philips conducted the survey to obtain an indication of managers' sleep habits, and their awareness of the impact of sleep on health and quality of life. Many people today do not sleep enough, and it is possible that the current economic climate has exacerbated that. We surveyed managers to obtain a focused snapshot of how one section of the workforce was sleeping. Sufficient sleep of managers is of critical importance for the workforce since managers not only need to perform their functional duties but also need to make sure their teams are reaching their objectives.

Methods. The survey was undertaken by TNS during March this 2009. It surveyed 2,513 managers – equally split across the UK (501), USA (502), Netherlands (501), Germany (500) and Japan (509) – using an online poll. 59% (1483) of the respondents were male and 41% (1030) were female. Age was distributed as follows: under 25 - 7%; 26 to 35 - 20%; 36 to 45 - 25%; 46 to 55 - 26%; 56 to 65 - 18%; over 65 - 4%. Only those with managerial responsibility were selected for participation. Distribution of occupancy was as follows: company owner / founder (31%); partner (5%); Board Director (4%); senior management (13%); middle manager/department manager (31%); and junior manager / supervisor / team leader (16%).

Results. 72% of the managers are sleeping 7 hours or less every night. 39% of those questioned blame the state of the world economy as the major reason for their lack of sleep. This was significantly less in Dutch managers; only 15% blamed the financial crisis for their lack of sleep. A majority of respondents to the survey (61% overall, 53% in NL) say they have had mainly their work impacted negatively by lack of sleep. On average, each estimated 6.2 days per year were impacted by inadequate sleep – costing companies around the globe millions. In the Netherlands for example, 6.1 days per year are impacted by lack of sleep and companies are losing nearly €1300 of productivity per manager per year. With 375.000 managers in the Netherlands, the cost to the economy could be as high as €500 million a year. Lack of sleep also negatively impacted family live (34% overall, 24% in NL), rated significantly lower than the effects on work. Most reported negative effects of sleep loss were difficulties with concentration (58% overall, 47% in NL), less patience (51% overall, 49% in NL) and less enthusiasm (49% overall, 36% in NL).
In addition to the findings about lack of sleep, the survey also found that while 96% (94% in NL) of managers recognize that inadequate sleep can seriously affect a person's health, only 29% discuss their problematic sleep patterns. Of those that do, just 27% (20% in NL) seek professional help from a physician with the majority (74% overall, 78% in NL) simply talking about their problems with family and friends.
22% of the managers reported to wake up during the night as cause of stress at work (12% in NL). In the attempt to try to fall into sleep again 42% will start tossing, turning and watching the clock (38% in NL), 27% will watch television or a DVD (17% in NL) and 25% will read a book (21% in NL).

Conclusion. This survey shows that 4 out of 10 managers blame the current economical situation for their lack of sleep. Moreover, the vast majority of managers are sleeping less than 7 hours per night. Managers are aware that inadequate sleep takes a toll on health and wellbeing and hampers corporate performance. Only very few managers discuss problematic sleep patterns with a physician. Most managers tend not to have a strategy to fall asleep after nocturnal awakening and just keep tossing and turning.
Respondents in The Netherlands sleep more soundly and are less worried. Only a minority sees the economical situation contributing to sleep issues. In addition, the Dutch are less concerned by stress at work and money worries.

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RESTLESS LEGS SYNDROME IN PARKINSON'S DISEASE: A NORADRENERGIC LINK?

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Introduction. An association between RLS and PD is suggested by several studies, however evidence is still limited and studies in large patient groups considering the full spectrum of PD are needed.

Methods. In 269 non-demented Caucasian PD patients, the four diagnostic criteria for RLS as described by the International Restless Legs Syndrome Study Group were administered by a personal interview taken by a RLS trained researcher. In patients with a diagnosis of definite RLS by fulfilling all four criteria, the severity of the RLS symptoms was assessed. Furthermore, in all patients, relevant motor and non-motor symptoms in PD were evaluated.

Results. Definite RLS was present in 11% (n=29) of the patients. In most of the RLS patients, RLS developed after diagnosis of PD (76%). Patients with RLS were more often female (69% vs 32%, $p<0.001$) but no other significant differences existed between PD patients with and without RLS. Within the PD patients with RLS, the RLS severity score correlated positively with PD severity, motor problems, motor fluctuations, depressive symptoms, daytime sleepiness, cognitive problems, autonomic symptoms, and psychotic symptoms.

Conclusions. This study shows that in a large Caucasian PD cohort, the prevalence of RLS is similar to that in the general population, although the dopaminergic treatment probably causes underestimation of RLS in this population. No direct relation was found between the presence of RLS and PD symptoms, although relations between the severity of both RLS symptoms, if present, and PD-related symptoms, was apparent. It is hypothesized that the noradrenergic system plays an important role in RLS probably through its connections with the diencephalic dopaminergic system.

EFFECT OF SENSORY INPUT ON RLS SYMPTOMS

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Introduction. Restless Legs Syndrome (RLS) is characterized by unpleasant and disabling symptoms at rest associated with an urge to move the limbs which are relieved by voluntary movement, at least as long as the activity continues. Besides that, a diversity of sensory input may reduce RLS symptoms. The goal of our study is to test the effect of sensory input on the (subjective and objective) severity of RLS symptoms.

Methods. We performed a Suggested Immobilization Test (SIT) lasting thirty minutes, in three different conditions with the order randomized between subjects. The first SIT started at 8.30 PM and between the consecutive SITs there was half an hour possibility to move. Condition 1 existed of a SIT without an external stimulus. In Condition 2 an afferent sensory stimulus was given; an electrical stimulus of the posterial tibial nerve the ankle which was adjusted until a twitch of the big toe was seen. As this latter movement gives an (gnostic sensory) input we designed an additional condition; In condition 3 the same stimulus intensity was given as in condition 2 but the stimulator was placed aside from the posterial tibial nerve to prevent the twitch of the big toe. Participants were randomized for receiving the stimulus on either the right or the left leg. At baseline and every five minutes until the end of every SIT (in total 7 times) , the overall restless legs discomfort was measured on a Visual Analogue Scale (VAS; 0-100 mm). The mean VAS score (VASm) and the absolute difference between VAS score seven and baseline VAS score (VAS7-BL) were calculated of each SIT. As objective measurement of RLS severity the Periodic Limb Movements during Wakefulness (PLMW) index was calculated. Friedman tests of related samples were performed to evaluate differences between the median VASm, median VAS7-BL and the median PLMW index of all SITs in the three different conditions. P levels with a value of less than 0.05 were considered significant.

Results. Eighteen primary RLS patients participated. The median VASm scores (25-75th percentile) for condition 1, 2 and 3 were 42 (18.8-63.5), 30.5 (16.5-61.3), and 39 (18.5-66.5) respectively (Friedman test, p= 0.831). The median VAS7-BL scores for condition 1, 2 and 3 were 47.5 (13.8-68.5), 26 (9.8-51.0) and 39 (13.8-57.0) respectively (Friedman test, p= 0.401). For the median PLMW index these were 11 (0.0-53.0), 8.5 (0.0-81.5), 36.5 (0-125.5) for condition 1,2 and 3 (Friedman test, p=0.076).

Conclusion. In this well-controlled study, we did not find evidence that electrical sensory input reduces the objective and subjective symptoms of RLS.

Acknowledgment:S. Overeem assisted in designing and analyzing the study.

LONG TERM FOLLOW-UP OF MELATONIN TREATMENT IN CHILDREN WITH ADHD AND CHRONIC SLEEP ONSET INSOMNIA

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Introduction. To assess long term melatonin treatment course, effectiveness and safety in children with attention-deficit/hyperactivity disorder (ADHD) and chronic sleep onset insomnia (CSOI).

Methods. Follow up of participants who previously participated in a randomised controlled trial on melatonin efficacy by means of a structured questionnaire for parents. Response rate was 93 % (94/101). Mean time to follow up was 3.7 years.

Results. No serious adverse events or treatment related co-morbidities were reported. 65% of the children still used melatonin daily and 12% occasionally. Temporal discontinuation of treatment resulted in a delay of sleep onset in 92% of the children. 9% of the children could discontinue melatonin completely because of improvement of sleep onset insomnia. Long term melatonin treatment was judged to be effective against sleep onset problems in 88 % of the cases. Improvement of behaviour and mood was reported in 71 % and 61 % respectively.

Conclusion. Melatonin remains an effective therapy on the long term for the treatment of CSOI in children with ADHD and has no safety concerns regarding serious adverse events or treatment related co-morbidity. Discontinuation of melatonin treatment usually leads to a relapse of sleep onset insomnia and in resuming melatonin treatment, even after several years of treatment.

Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG. Long term follow up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. J Pineal Res. 2009. In press.

The results of this study were presented in poster session at the 19th Congress of the European Sleep Research Society in Glasgow, september 2008.

EXOGENOUS MELATONIN FOR DELAYED SLEEP PHASE SYNDROME: A META-ANALYSIS

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Introduction. To conduct a systematic review of the efficacy and safety of exogenous melatonin in advancing sleep-wake rhythm in delayed sleep phase syndrome.

Methods. This study evaluated those studies where circadian timing was measured or accurately predicted before treatment, which consisted of double blind, cross over or parallel administration of melatonin or placebo in patients with delayed sleep phase syndrome. PubMed, Embase and sleep and chronobiological societies abstracts (1990 – 2008) were searched for papers. The efficacy review included randomised controlled trials that involved humans with delayed sleep phase syndrome; written in English language; compared melatonin to placebo; and reported one or more of: endogenous melatonin onset, sleep onset, wake up time, sleep onset latency and total sleep time. Exclusion criteria were reviews and case reports, trials with other patient groups and/or other indications, trials with other treatment or combinations, and studies using other outcomes (like quality of sleep or life, biochemical measurements).

Results. Five trials including 91 adults and four trials including 226 children showed that melatonin treatment advanced mean endogenous melatonin onset with 1.18 h. (95% C.I. 0.89 to 1.48 h.) and sleep onset with 0.67 h. (95% C.I. 0.45 to 0.89 h.). Melatonin decreased sleep onset latency with 23.27 min (95% C.I. 4.83 to 41.72 min.). Wake up time and total sleep time did not change significantly.

Conclusions. Melatonin is effective in advancing sleep-wake rhythm and endogenous melatonin rhythm in Delayed Sleep Phase Syndrome.

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SLEEP AND THE RELATIONSHIP TO MOOD IN A HEALTHY ADULT SAMPLE

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Introduction. Mood depends on different kinds of events and impressions. We hypothesize that a person's mood is related to the sleep of the night before. Studies that found evidence for this hypothesis often rely on single measurements of mood, whereas mood may fluctuate during daytime. Therefore, we measured mood throughout the day. In addition, most studies investigated between-subject effects, whereas we also studied within-subject effects to increase the study's reliability. Following people for more than one night and day combination allows to control for individual differences, for example in sleep need.

Methods. Twenty-four participants (9 female, aged 30-55 years, without sleep problems, no shift-workers) took part in the study which lasted one week, including weekend days-off. During the night participants wore a SenseWear PRO3 Armband. Each morning the participants filled out a sleep diary. Eight times a day a mood survey was completed on a PDA. The independent variables TST, SOL, and WASO were measured both objectively (SenseWear) and subjectively (diary). Subjective Sleep Quality (SQ) was rated on a seven-point scale, ranging from very bad to very good. Mood was measured using six bipolar mood items (e.g., agitated-calm). Mixed linear models were used to analyze the data both between-subjects and within-subjects.

Results. Subjective and objective measurements of the same sleep parameter correlated, but were not the same. Above all, day-to-day variations in subjective SQ, TST, and SOL affected mood. Between subjects, SQ and WASO related to mood. On the contrary, objective measurements practically did not relate to mood. Furthermore, sleep quality was better during nights preceding a day off than during nights preceding a working day.

Conclusion. A person's sleep affects day-to-day fluctuations in mood, despite all kinds of events and impressions that also influence mood during the day. Further, we found that subjective perceptions of sleep are more important for mood than objective parameters.

Presented at the 11th Annual international clinical symposium Kempenhaeghe
Presented at SLEEP 2009 23rd Annual meeting of the Associated Professional Sleep Societies (APSS).

USING QUALITATIVE METHODS TO STUDY SLEEP IN HOME CONTEXTS

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Introduction. A vast amount of the studies conducted with the aim to improve sleep focus on a population with clinical conditions or sleep disorders. However, many people without consistent sleep complaints also desire to improve their sleep. This study aimed at understanding issues surrounding sleep in healthy people's lives.

Method. A context mapping study was set up, supplemented by focus groups, both qualitative methods. Context mapping studies, conducted in participant's homes, provide participants with tools to express their routines, experiences and worries. The focus groups served to increase the validity and reliability of the context mapping findings. Prior to the home visits and focus groups, all participants (aged 30-55 years, with a busy lifestyle, wanting to improve their sleep) completed a booklet with exercises about their relaxation behavior, sleep processes and disturbances. The context mapping study involved 7 couples in the Netherlands. The focus groups involved 32 (4 x 8) English participants.

Results. We identified several common sleep routines, experiences, and environmental and psychological sleep thieves. First, many people experience problems sleeping with even the smallest amount of light. Second, every little noise alerts them. Third, cold feet are detrimental for falling asleep. Fourth, many people have difficulties switching off their minds before sleep. Counter-measures include distraction by watching TV, preparation for the next day, and practicing relaxation exercises. Fifth, especially women have difficulties sleeping due to an 'unsafe' feeling such as the worry about potential burglars. Sixth, people prefer waking up 'naturally' without an alarm. No major differences between the nationalities were found in sleep routines, experiences, and thieves.

Conclusion. Context mapping and focus groups appeared useful qualitative methods to gain insight into sleep of healthy people in home contexts. Further, the results showed that even people without consistent sleep complaints would like to improve the quality of their sleep.

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FACTORS RELEVANT FOR PERSISTENCE OF RESTLESS LEGS SYNDROME AFTER KIDNEY TRANSPLANTATION

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Introduction. Restless Legs Syndrome (RLS) is prevalent in uremia and in dialysis patients. Symptomatic therapy is similar to that in non-uremic patients. Real cure is reported in most post-transplantation patients. Exceptions are possible, but rare. The aim of the study is delineation of factors prohibiting cure of RLS after transplantation.

Methods. Two male patients (53 and 60 y/o) still complained about severe RLS despite transplantation, requiring continuation of dopa therapy. Factors which might be of relevance for the unchanged situation were analysed

Results. The primary disorder was kidney cysts (patient A) and glomerulonephritis (patient B). They were treated with hemodialysis and peritoneal dialysis respectively. Co-morbidity was apnea syndrome (AHI: 35), morbus Bechterew, slight depression, psoriasis and atrial fibrillation in patient A and aortic stenosis, polyneuropathy and slight depression in patient B. Both patients had severe RLS before transplantation. Ferritine levels were normal. Patient A underwent a living donor procedure in 2007; patient B received a kidney from a brain-dead donor in 1991 and had a repeated, living donor transplantation in 2005. The transplant functions were estimated at 55% for patient A and 70% for patient B. Patient A had no improvement in his RLS symptoms (IRLS score: 21) at all and needed dopamine agonist therapy. Patient B was free from RLS between 1991 and the retransplantation procedure. After the second operation RLS recurred as severe as before 1991 (IRLSS 34). RLS therapy was started. Both patients were on a triple immunosuppressant regime with cyclosporine, prednisolon and MMF. Patient A had CPAP as well; both patients needed a SSRI antidepressant.

Conclusion. Factors which may be important and are common for both patients, are a living donor transplantation and the use of similar immunodepressant regimes and SSRI's for depression. We postulate that all three factors are important with emphasis on the transplantation procedure that was chosen.

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