SLEEP-WAKE
Research in The Netherlands

Annual Proceedings of the NSWO
Volume 21, 2010

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PREFACE

Traditionally, the main focus of this preface is on some of last years' highlights among the activities of our society. However, since I have served my term and on March 26th this year stepped down as president (and thus am writing 'in spare time'), I have taken the liberty to deviate from this tradition and share some personal thoughts with you on the future of the NSWO.

More and more, the topic of Sleep is drawing the attention of already established fields of expertise, e.g. disciplines such as molecular genetics, cognitive sciences, dentistry, pulmonology, neurology and other medical sciences. Specific, discipline-oriented scientific journals publish increasing numbers of articles that report on sleep research. This is an encouraging development, as it reflects a growing broadening of the recognition that sleep has a major impact on many, indeed if not all, aspects of life.

In my opinion, there is reason for some concern, however. The study of Sleep, at all possible levels of analysis - from the dynamics of single cells, through the brain systems involved, the development and acquisition of human sleep behavior, its formal and computational modeling, to the pathological and therapeutic aspects of sleep disorders -, is of an intrinsically interdisciplinary nature. Major progress can only be made by integrating already existing fields of expertise into one interdisciplinary effort.

Lately, parallel to the recently increasing number of sleep-related papers in many different scientific journals, we witness the emergence of discipline-specific societies focusing on sleep. Examples are sleep medicine societies within the framework of disciplines like dentistry, neurology and pulmonology. Although I welcome the formation of such expert groups, there is a risk that the interdisciplinary nature of sleep research and sleep medicine is pushed into the background and does not get ample opportunity to develop itself into an interdisciplinary science.

The NSWO is in an excellent position to cross disciplinary borders and to offer an interdisciplinary, federative platform for all these discipline-specific sleep societies and their members. To this end, it should actively approach these disciplinary sleep groups and explore the possibility of a federal association. In concerted action it should define interdisciplinary themes and organize expert meetings and workshops, with the intention to stimulate the exchange of ideas, formulate research plans and grant proposals, draw up consensus reports for clinical applications, etc. In addition, the NSWO should actively involve the different disciplinary sleep groups in formulating programs for (inter-)national teaching courses. In this way the NSWO can stimulate the development of sleep research and sleep medicine into a truly interdisciplinary science of sleep.

Gerard A. Kerkhof, past-president

EDITORIAL NOTE

Before you lies the 21st edition of the proceedings of the Dutch Society for Sleep-Wake Research. In this edition two distinguished researchers, Serge Daan and Hans Folgering present their personal reflections on their contribution to sleep research.

Ellemarije Altena presents the content of her PhD thesis which she defenden in the past year. We are much honored that Pierre Maquet was willing to provide a comment on the thesis of the new PhD.

From the beginning the proceedings were intended to provide young sleep researchers in the Netherlands an opportunity to publish their work in an easy accesible medium, and to give a broad overview of sleep research performed in our country. However, research is an international undertaking and this is reflected in the contributions. I am very happy that, already for several years, the proceedings also contain contributions from Dutch sleep researchers working abroad.

Similar to last year not only mini-papers, but also research abstracts of work of the past year, published or in press, are included. After a mini-review by Tom Coenen about the history of the electroencephalogram, you will find fourteen mini-papers, followed by 36 abstracts. On behalf of the scientific committee, I would like to thank all NSWO members for their contributions. In addition, from my side many thanks to my co-editors for reviewing the manuscripts, ensuring the highest quality possible.

The proceedings are completed by an updated member list. This list, together with a lot more information about sleep research and sleep medicine in the Netherlands is also available on the NSWO website (www.nswo.nl).

I wish you interesting reading!

Leiden, September 2010

Tom de Boer

Chair Scientific Committee
Chief Editor NSWO Proceedings

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Special reviews

THE MARRIAGE OF SLEEP AND RHYTHMS RESEARCH

A personal reflection on three decades of collaboration between Zürich and Groningen

Serge Daan

Niko Tinbergen distinguished honorary chair,University of Groningen

Sleep research and the study of biological rhythms were once separate domains. The approaches and questions were very different. One research field focused on sleep, the other on wakefulness. Sleep research was organized around electroencephalography. The technique, introduced in the 1930's, had opened up the quantitative analysis of sleep. With the discovery of REM-sleep by (Aserinsky and Kleitman 1953), patterns within sleep became accessible. One asked questions on the fundamental processes within sleep – then and now the only dominant behaviour of which the primary function is unknown. In biological rhythms research, not the state of wakefulness or that of sleep, but the timing of their alternation was the key problem. The prominent technique was the recording of spontaneous activity in undisturbed isolation. The two research traditions, although concerned with intimately related phenomena, spoke in different tongues. The language of sleep stages and spectral EEG analysis in one, the language of oscillators, of phase and period and amplitude in the other. There was little crosstalk. Both fields were blossoming in the 1960's and '70's, but their flowers were not crossfertilized. This came to an end in 1980 at the Ringberg Conference on Vertebrate Circadian Systems.

Of course there had been overlap before. The sleep pioneer Nathaniel Kleitman himself had been interested in daily rhythms, and done pioneering experiments with humans living on a 28 h schedule for 32 days in the Mammoth Caves in Kentucky. In Jürgen Aschoff's lab at the Max Planck Institute for Behavioural Physiology in Andechs, Bavaria there were visitors from nearly every branch in the life sciences, including occasional sleep researchers. After my Ph.D. work on hibernation at the University of Amsterdam, I was a postdoc in that institute from 1971 till 1973. I recall participating in a sleep project as a subject. The study aimed to test if the first REM episode is tied to sleep onset and can be moved to different clock times by going to bed at different times. (There were no instructions concerning alcohol consumption, and I disqualified when after too much wine one night I fell asleep while the electrodes were mounted, and skipped the first REM episode). But no sleep researchers participated in any of the classic biological rhythms meetings at Cold Spring Harbor (1959), Feldafing (1965), and only one in Friday Harbor (1971).

In the late 1970's rhythms researchers slowly opened their eyes to sleep. This interest was stimulated by the development of human circadian rhythms research, in particular in Aschoff's own institute. 'Bernie' Webb contributed a chapter to the bible of rhythms research, the Handbook of Behavioural Neurobiology vol 4 (Aschoff 1981). When Jürgen Aschoff, Gerard Groos and I in 1980 planned the Ringberg meeting, we decided to invite Alexander Borbély to participate. That was a stroke of luck.

I had never met Alex before, but he and Anna Wirz-Justice had sought contact three years earlier, in 1977. They invited me to a meeting with a group of psychiatrists and sleep researchers in Romainmotier, Switzerland. My five papers with Colin Pittendrigh on 'A functional analysis of circadian pacemakers in nocturnal rodents' had appeared in 1976 (Pittendrigh and Daan 1976), and apparently made me a rhythms representative attractive to sleep researchers. But I was not yet ripe for the challenge. I had just returned to Holland from Stanford University and accepted a tenured position at the University of Groningen. I was building up a research program on the functional significance and fitness consequences of timing, and wanted to initially retain my focus. So I declined Alex' invitation. Inviting him to Ringberg three years later was the best way to make up for this foolish mistake.

Ringberg is a peculiar castle in the foothills of the Alps in southern Bavaria. It is a both ugly and romantic fortress, built in the 20th century by the duke Luitpold of Bavaria and his intimate friend Friedrich Attenhuber. When the duke died in 1973, he left it – still unfinished - to the Max Planck Society. It was decided to turn Ringberg into a meeting place for scientists. Aschoff was among the first to use it as such. He had tried out the castle in the summer of 1976 with a "*Sängerfest*" (Singing party) for the members of his institute, and thought it convenient for another international meeting on biological rhythms. The meeting was restricted to Vertebrate Circadian Systems, and included a substantial number of students of human rhythms.

In those days, internal desynchronization of circadian rhythms in humans (Aschoff 1965) had become well established. In prolonged isolation from time cues a subjects often displayed a rhythm of sleep and wakefulness with a period much longer than usual, varying around 34 hours (Wever 1979). This rhythm shows relative coordination with the circadian body temperature rhythm that retains its common cycle length of slightly over 24 h. The generally accepted conclusion was that there must be two endogenous circadian oscillators or pacemakers in the body with different functions (e.g., (Wever 1979), (Kronauer 1982), (Gander et al. 1984)). Only one was known, the *suprachiasmatic nucleus*. Yet some explicit hypotheses on misalignment of the two oscillators in mood disorders were considered (*e.g.,* (Wehr and Wirz-Justice 1981)).

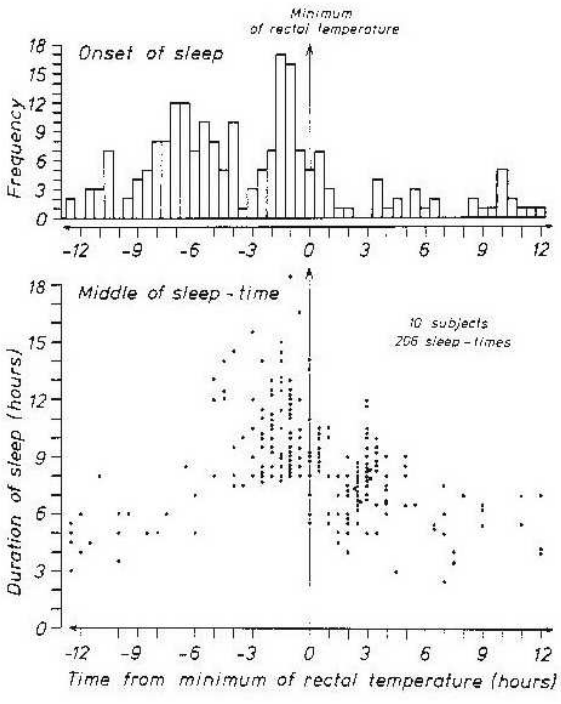

Figure 1. Circadian distributions of sleep onset in subjects with internal desynchronization (upper panel) and of subsequent sleep durations (lower panel. From (Zulley and Wever 1982).

I remember vividly what happened during those early October days of 1981 at the Ringberg Schloss. I had given the opening lecture on functional significance of circadian rhythms, and quite relaxed enjoyed the other talks. The first thing that happened was Charmane Eastman presenting her unorthodox view on the dual pacemaker system (Eastman 1982). She proposed that internal desynchronisation could be explained with a single pacemaker controlling body temperature as well as sleep, but with a periodically skipped sleep episode. The idea was good, but her

proposition did not specify why sleeps were skipped.

The next event was the talk of Jürgen Zulley, then collaborator of Rütger Wever at the Max Planck Institute in Andechs. Zulley had analysed the duration of sleep episodes in a series of subjects who had shown internal desynchronization in the bunker. He plotted these as a function of the phase in the circadian temperature cycle when the sleep episode started. The curious graph showed two concentrations of points, one with sleep starting about 6 h, the other about 1 h before the minimal body temperature. The first cluster led to long sleeps (ca 10 h), the second one to ca 8 h sleeps (Figure 1). It was as though there were two preferred circadian phases for the onset of sleep. The longer sleep in the first group might be due to long preceding wake times (skipped sleeps) and also to the influence of the circadian system that would terminate both sleeps at about the same time and phase. An intriguing pattern that remained unresolved.

Then came the presentation of Alex Borbély. He introduced a model of a sleep dependent – homeostatic - process that was based on the EEG slow wave power density and that interacted with a threshold with rectangular daily variations controlled by a circadian oscillator (Figure 2). The timing of sleep onset and end in this model were always dictated by the instantaneous drop and rise of the threshold. Listening to Alex' inspiring talk it suddenly dawned upon me that if the threshold was sinusoidal rather than rectangular and of lower amplitude, his model might explain internal desynchronization: In constant conditions, the sleep onset might occasionally be missed when the rising curve remained below a threshold. The subject would then expose itself to spontaneous sleep deprivation. This could be Eastman's missing sleep phase, and it would immediately explain Zulley's curious clusters of sleep duration. I was very excited and immediately discussed the possibility with Alex and Anna Wirz-Justice over lunch. I had never met either of them before, but there was an immediate feeling of mutual interest, indeed of friendship. A friendship that would not abate for the rest of our life.

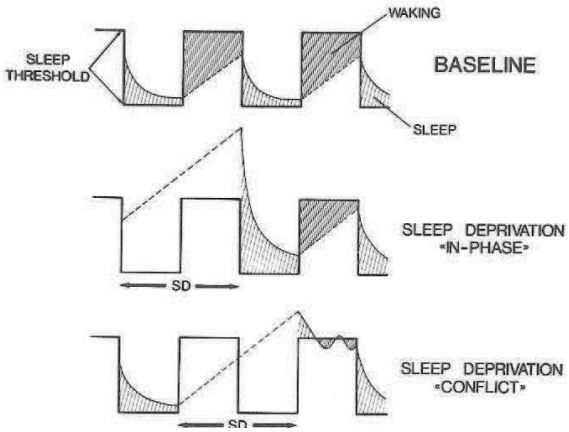

Figure 2. The preliminary version of the two-process model as discussed by Borbely at the Ringberg meeting. From (Borbély 1982b).

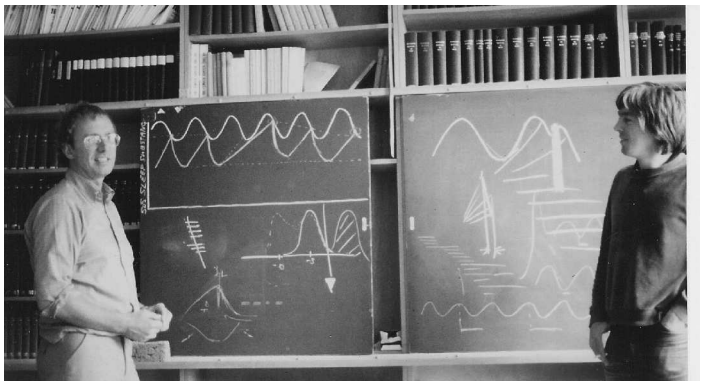

Figure 3. Serge Daan (left) and Gerard Groos with the upper threshold model. Library of the MPI Verhaltensphysiologie, Andechs 4.10.1980. (Photograph Anna Wirz-Justice).

After the Ringberg meeting several of the attendees moved to the Max Planck Institute in Andechs. I asked Aschoff to allow me to give a seminar on a new model for internal desynchronization the next day, October 4, 1980. Ben Rusak was there, Charmane Eastman, Rütger Wever, Gerard Groos, and Anna Wirz-Justice, who took a picture of Gerard and myself outlining the model in the library of the institute (Fig.3).

I went home greatly excited. Eastman was right: internal desynchronization did not require a second pacemaker. It required only the much simpler and natural need-need reduction process proposed by Borbély. I was also excited because at Ringberg I had met Ruth Hohe, my later wife. I had missed my friend Joost Tinbergen's Ph.D. thesis defence in Groningen, but the meeting had been worth the loss in many ways.

Back home, things developed fast. Two weeks after Ringberg I received a phone call from Domien Beersma, a biophysicist working in Biological Psychiatry, Rudi van den Hoofdakker's department in Groningen. He had attended, a week after Ringberg, the European Sleep Research Conference in Munich, where Anna Wirz had told him that *"Serge Daan has a model that explains everything"* (Domien later gave me his sticky note with this text). We got along well, and decided to generate computer simulations to show that one could indeed simulate internal desynchronization. Alex Borbély generously sent his then unpublished data on the effect of sleep deprivation in human subjects on the EEG power density (Figure 4). These were crucial to estimate the rate constants of the buildup and breakdown of process S. (Åkerstedt and Gillberg 1981) had measured spontaneous sleep duration after 4, 8, 12 … 24 hrs of wakefulness in 6 Swedish subjects and these yielded the circadian threshold variations.

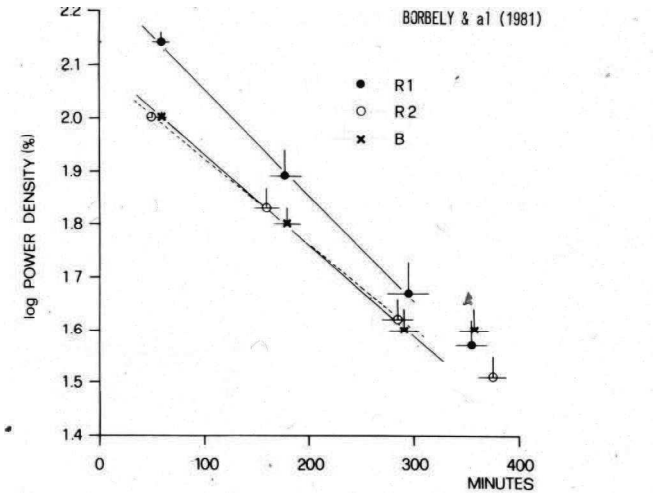

Figure 4. The exponential decay of slow wave activity during sleep. From (Borbély et al 1981).

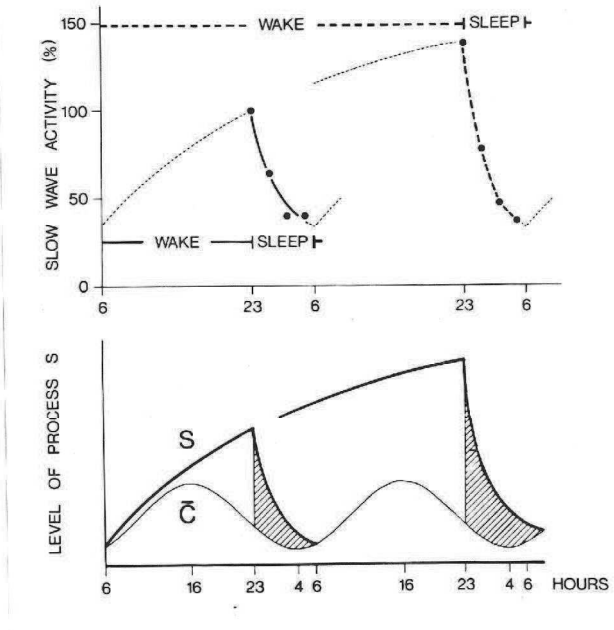

Figure 5. The two process model as presented by Alex Borbély in Hyannis, June 1981. From (Borbély 1982a).

The rumour of the new model spread. In October I received an invitation from Martin Moore-Ede and Charles Czeisler to attend a meeting in June 1981 on Mathematical Models of the Circadian Sleep-Wake Cycle. The condition was that I should submit a finished manuscript before the meeting. This would be published that same year in a symposium volume by Raven Press. Domien and I worked hard that winter, and I did bring the manuscript to Hyannis, Cape Cod in June. It seemed the optimal place to get the paper out fast. In Cape Cod, I met Alex Borbély again. He gave an impressive keynote lecture to the APSS on his model. The model was much advanced compared to the Ringberg version. By now he used a sinusoidal threshold, and called this process C (for circadian). We had lots to discuss. Process C in Alex' model at that stage was the lower threshold, which would predict variations in wake-up, not in sleep onset (Fig. 5). In my original version drawn on the blackboard in Andechs in 1980

(Fig.3), I was concerned with an upper threshold, which would predict the onset, not the end of sleep. To model circadian behaviour in a time-free environment, we needed both thresholds, an upper and a lower one. That was realized in the computer model written up with Domien Beersma.

Alex told me in Cape Cod that he was preparing a manuscript based on his lecture, and we were confident that both our versions of the two-process model would appear soon and simultaneously. His work was published in the first volume of the new Springer journal Human Neurobiology (Borbély 1982a). Our manuscript rested with the editors of the modeling symposium, Moore-Ede and Czeisler, for two years. When at long last it appeared in print (Daan and Beersma 1983), in November 1983, Raven Press stamped 1984 on it as the year of publication – to make the book look more recent, although the text was three years older and totally out of date.

Following the Cape Cod meeting, Alex and I intensified contact. Anna Wirz-Justice strongly stimulated and participated in the collaboration. In the weekend of September 5, 1981 she organized a busy and inspiring little workshop in Nothweiler, the cottage of the Aschoffs in the Pfalz, right on the French border. It was a beautiful Indian summer weekend. The Aschoffs, as always, splendidly hosted us, Alex, Anna, Domien, Gerard Groos, and me. We realized that the complete, two-threshold model was applicable to many situations outside the realm of the bunker and of internal desynchronization. Alex and Anna extended the model to the special situation of depressive illness in a second paper (Borbély and Wirz-Justice 1982). But there was more: shiftwork, continuous bedrest, self ratings of fatigue, *etc.* Hence Alex, Domien and I decided to integrate all of these datasets around in the literature, simulate them with our model on the computer, and write a comprehensive essay on gating of sleep, that covered a broader and more general area of both chronobiology and sleep research. Alex came to Frankfurt on January 2, 1982 where he and I spent a day together outlining the new paper. A new burst of programming and simulation by Domien started. In March 1982 Alex hosted the European Sleep Research Conference in Zürich. It was preceded by a satellite symposium on models, and followed by an informal workshop, run by Anna Wirz-Justice in another romantic country setting, in Romainmotier, Switzerland. Alex and Anna invited me to both. It was a thrill to experience the great interest the model by now attracted. And a bit of irritation: in Zürich Ian Feinberg told the audience that he had to exhume his old model (Feinberg 1974) which, he said, had preceded Borbély's.

I drafted the joint paper in Seattle in the fall of 1982. I was there both for a sabbatical, with Jim Kenagy, thanks to a Fulbright fellowship and on honeymoon. We submitted the manuscript to the American Journal of Physiology in 1983, and it appeared the next year (Daan et al. 1984). It is interesting to see what happened to these papers. The Web of Science finds 1086 citations of the (Borbély 1982a) paper (June 2010). It surely is one of the most cited papers in sleep research, with the annual number of citations still growing every year. The Cape Cod paper (Daan and Beersma 1983), with the first full version of the model, is reported to have been cited 46 times. The AJP publication in 1984 (Daan et al. 1984) made it to 516 citations. The lesson is clear: never entrust one of your lifetime ideas to a symposium volume. But Alex and I were never so much interested in priority and citations. We felt that both of us had contributed to the marriage of sleep research and chronobiology. He had contributed the idea of sleep as a homeostatic process reflected in the EEG, and the proposition that this is somehow controlled by the circadian system. I had suggested the idea of a sleep onset threshold that would explain bedtimes in the bunker experiments. Domien Beersma and I developed the rigorous quantification of the interaction, and showed that there is no need for a second circadian pacemaker. The three of us were excited about the elegance of the theory, and its applicability to understanding what may be the most prominent and

robust human behaviour, the alternation of sleep and wakefulness. We went on researching this, in close collaboration.

The theory met with great enthusiasm of some researchers, and with resistance from others. Art Winfree belonged to the first group. Art had been at the Cape Cod meeting in 1981, and presented a model of a circadian wake-up threshold to explain the bimodality of preferred circadian phases of awakening. Shortly afterwards, he was asked by the United States Air Force to provide a detailed report on available theories of human circadian sleep regulation. This request followed the debacle of the Operation Eagle Claw on April 24, 1980. This was the attempt to free 52 US hostages in the American Embassy in Teheran. After a series of mistakes made by the liberators in the deep of the night, president Carter had to cancel the mission. The army became curious about the circadian regulation of human sleepiness and efficiency. Winfree compared all available theories and summarized: *"Daan and Beersma's two-threshold model is the best product on the market at present"* (A.T.Winfree, private communication, November 1982). It felt as though we might have successfully liberated the hostages.

Others were less positive. In 1982 several papers from the Harvard group appeared with elaborations of what may be called Kronauer's two pacemaker model. Martin Moore-Ede published a study with the title: "…. *Two pacemakers preside over many secondary oscillators*" (Moore-Ede 1983). In 1984, Alex Borbély received funds from the Neuroscience Institute to set up a meeting between the mainly European proponents of the two-process model and the Harvard group. He asked me to coorganize this. We met in New York at Rockefeller University on February 21, 1985. Dick Kronauer, Charles Czeisler, Philippa Gander, Steve Strogatz, sat at one side of the table, Alex Borbély, Domien Beersma, myself at the other side. A few others without stakes in the debate also attended: Tom Wehr, Chris Gillin, and Michael Terman. We discussed the question of one or two pacemakers essentially for a whole day. The somewhat nebulous picture, taken on the roof of the building (Fig. 6) does not reveal that neither party became convinced by the other. Nonetheless, a publication of the Harvard group appeared a year later entitled *"Bright light resets the human circadian pacemaker*" (Czeisler et al. 1986). It did not mention the possibility of a second pacemaker, or acknowledge the discussion we had had. Later publications from Harvard never referred to the elusive second pacemaker anymore.

Figure 6. Participants of the meeting in New York, February 21, 1985: from left: Dick Kronauer, Chris Gillin, Michael Terman, Alex Borbély, Charles Czeisler, Philippa Gander, Tom Wehr, Serge Daan, Steve Strogatz, Domien Beersma.

The model became exceedingly popular. I was invited to countless seminars and meetings to talk about it, such as the Gordon conference on Chronobiology (1983, 1985), the World Congress of Psychiatry (Vienna 1983), the International Ethological Conference (Toulouse 1985), the Sapporo Symposia on Biological Rhythms (1986, 1991), the First International Congress of Neuroethology (Tokyo 1986). Meanwhile at home, we were not idly enjoying the success. In Zürich, the model became the

main source of inspiration, and further research of both Alex Borbély and Irene Tobler and their students soon made their lab the leading place for sleep research in Europe, if not the world. In the summer of 1982, preceding my sabbatical, Domien Beersma and I wrote a large grant proposal to actively test a series of predictions that emerged from the model. I submitted that grant to BION, the branch of NWO that covered the life sciences. In those days, BION was organized in 'werkgemeenschappen' (working communities). In these bodies the priority of proposals was decided collectively. Mine was in the werkgemeenschap Ethology. After my presentation in Utrecht on April 6, 1982 my ethological colleagues hammered the proposal down, with the main argument that this was an interesting idea, but did not belong in ethology. When the verdict was almost clear, Professor Gerard Baerends arose. He was the grand old man of ethology in the Netherlands. He never said much in meetings, but sat listening silently in the back row. But this time his fury was unleashed. What he said in essence was that ethology would not be worth its existence if we thought that the most prominent of human behaviours were not our concern. Silence. In the subsequent vote, the proposal came out with top priority. The grant was awarded. Rudi van den Hoofdakker, professor of Biological Psychiatry, in whose department Domien worked at that time, would act as the thesis supervisor. Rudi was working intensely on sleep deprivation therapy in mood disorders. He was a cofounder of the European Sleep Research Society, and strong supporter of our endeavour. We hired Derk Jan Dijk to make a Ph.D. out of it, Gerda Bloem to assist him technically. Derk Jan set out towards a splendid thesis, defended in 1988 with the highest honours: *cum laude* (Dijk 1988). He was the first progeny of the marriage. Alex Borbély was on the committee (Fig. 7).

Figure 7. Alex Borbély at the party following the thesis defence of Derk Jan Dijk (left), June 22, 1988.

Groningen and Zürich further tightened the bond. Alex and I received a grant from the ETP-BBR (European Training Program in Brain & Behaviour Research). This started a series of mutual two-day visits, initially twice per year, in which our groups exchanged and discussed our work with each other. Collaboration and friendship between the Groningen and Zürich/Basel groups became ever more intense and rewarding. This continued all through the 1980's and 90's. After his successful thesis defence, Derk Jan Dijk went for a postdoctoral fellowship to Zürich. Later two of our undergraduate students in Groningen went to the Borbély lab for their thesis research: Paul Franken, who extended the quantitative modeling work to rats, and Tom de Boer, who was involved in an important functional sideline of circadian sleep research: the relation between sleep and torpor. Both have meanwhile made a career of their own.

I had not touched the problem of torpor anymore since my own Ph.D. thesis (Daan 1973). That thesis was focused on the question why hibernators periodically warm up from hibernation during the winter. That was an important biological problem. Many hibernators do not eat or drink during the winter, but live on a massive fat resource laid down during the summer months. They use nearly all of this fat in the periodic rewarming for about 10 hours once every 10 days or so. I had found that bats use these so-called 'arousals' to relocate in

their hibernacula, selecting the coldest spots: deep in the cave in the fall, in the front sections later in winter. But this could not be a general function since most other hibernators stay where they are for six months. On May 5, 1989 Jim Kenagy from at the University of Washington, who had hosted my sabbatical 6 years before, visited us in Groningen together with a former and a current Ph.D. student, Brian Barnes and Sarah Hiebert. Sarah gave a beautiful seminar on her work on hummingbirds. She showed daily profiles of their body temperature, and it struck me that hummingbirds always warmed up from nocturnal torpor an hour or so before lights-on. This seemed a waste of energy for such a small creature. That night, sipping wine on our terrace with our american visitors, I suggested that hummingbirds might warm up from torpor in order to sleep before becoming active. Perhaps hibernators too would periodically warm up to sleep. This would solve the question I had left unanswered two decades earlier.

Another wave of excitement started. We had the EEG equipment for small mammals. Brian Barnes, at the Institute of Arctic Biology in Fairbanks, Alaska, had the animals. He sent a batch of Arctic ground squirrels over to Holland. My student Arjen Strijkstra recorded their EEG during hibernation. Indeed the squirrels slept soundly during every "arousal", with high-intensity slow wave sleep. When we had written up the first part of the story, it turned out that Lorenz Trachsel had found the same result in a close relative, the Golden-mantled ground squirrel. Lorenz was a former Ph.D. student of Irene Tobler and Alex Borbély, holding a postdoctoral fellowship in Craig Heller's lab at Stanford University. The publications came out simultaneously (Daan et al. 1991), (Trachsel et al. 1991). We were in high spirits indeed. Not because the old riddle was finally solved, but since the result was of major significance for the elusive function of sleep. If animals decided to systematically interrupt their major energy saving and life-prolonging strategy of hibernation in order to sleep, and indeed burn their fat for sleeping, sleep could not have an energy saving function as was a popular speculation (Berger and Phillips 1988).

Arjen Strijkstra embarked on a Ph.D. project on this phenomenon (Strijkstra 1999). He used European ground squirrels, or susliks, as they are called in Hungary. We had access to them through collaboration with John Dittami and Eva Millesi at the University of Vienna. We further revived active collaboration with Zürich (Irene Tobler) and with an old friend from the Andechs days: Gerhard Heldmaier, who carried on the torch of Max Rubner at the University of Marburg. A new grant from ETP-BBR supported our collaboration and exchange, and a series of meetings in Groningen, Zürich and Marburg. Tom de Boer did his Ph.D. research with Irene Tobler in Zürich on sleep and torpor in the Siberian hamster, and eventually defended his thesis in Groningen in 1996 (De Boer 1996). Arjen showed for hibernation, and Tom for daily torpor, that brain temperatures below 30^{o}C constitute sleep deprivation and have to be compensated by slow-wave sleep afterwards. This was a very important sequel to the two-process model, since it demonstrated that Borbély's sleep need builds up not only during wakefulness but also during low brain temperatures. The torpor work also invigorated the interaction between the two labs in its second decade.

On neither side the collaboration was our only activity. Alex and Irene, with the help of Peter Achermann went on refining the model (Achermann et al. 1993; Borbély and Achermann 1999) and had a very active crew strongly focused on the sleep EEG. Their many Ph.D. students have spread over the world and changed the face of sleep research. Among them was an other Groningen student, Paul Franken, who was the first to apply the two-process model systematically to an animal species, the rat (Franken 1993). In Groningen, I was appointed in 1996 in Gerard Baerends' chair of Ethology, soon afterwards relabeled as Behavioural Biology. There were 3.5 tenured scientist positions in the group, and our work covered a broad variety of topics, including energetics, reproductive timing, sex ratio

optimization, perinatal effects on adult behaviour. Circadian rhythms and their entrainment remained a strong focus especially after the recruitment of Domien Beersma (1997) and Martha Merrow (2004) to the tenured staff. It will remain even more so with the recent appointment (2010) of Roelof Hut. In 2005, when I stepped down as group leader, the unit had grown to 7 tenured scientists and we decided to split it into Behavioural Biology, headed by Ton Groothuis and Chronobiology, lead by Domien Beersma.

Figure 8. Alex Borbély (left), Serge Daan (right).

While our labs in Zürich and Groningen continued the interaction in the last decade, both Alex and I were called upon for administrative duties. Alex was dean of the Medical Faculty (1998-2000) and vice-rector for research at the Univerity of Zürich from 2002 to 2006. I was vice-dean (2001-2004) and dean (2007-2009) of the Faculty of Mathematics and Natural Sciences at the University of Groningen. While we were fading out, the integration of sleep and rhythms research still flourished. Sleep had become a topic in most of the leading biannual conferences (SRBR, ESRS, SLTBR, Gordon Conference on Chronobiology). Our labs continued to interact. Our students settled in leading positions. Derk Jan Dijk, in his Harvard time with Charles Czeisler, had beautifully shown by forced internal desynchronization how the contributions of the two processes to psychophysiological rhythms can be disentangled (Dijk et al. 1992). Special symposia were dedicated to our contributions, in Tokyo 2006, in Ittingen 2007. A new generation of researchers has started to fill in what exactly the two processes are. Great strides have been made in unraveling the molecular mechanism of circadian oscillations. For the homeostatic process a challenging new hypothesis is now pursued: the synaptic downscaling role of sleep proposed by (Tononi and Cirelli 2006). What more could we have wished to happen ?

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EARLY DAYS OF SLEEP AND BREATHING IN THE NETHERLANDS
Some personal experiences

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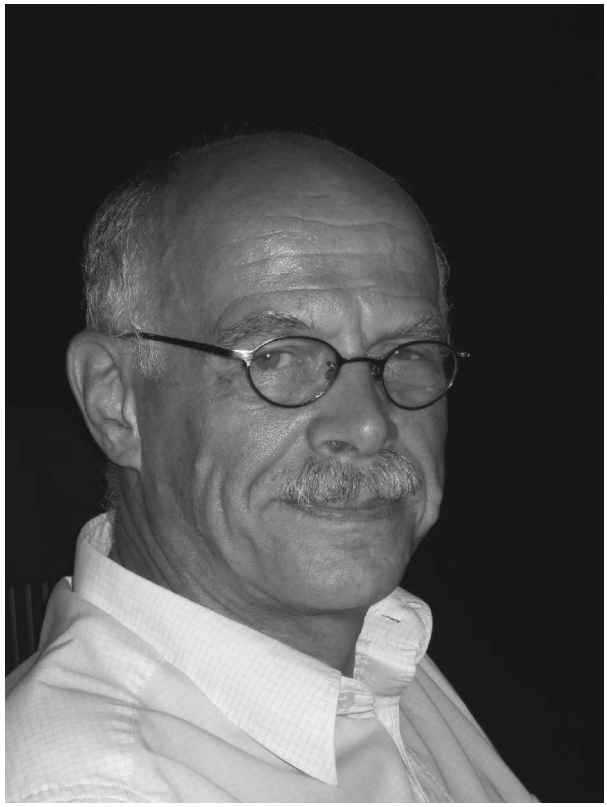
SUMMARY CURRICULUM.



As a medical student, I was invited to work in the department of medical physics for three years (1963-1966) on oximetry in hemoglobin solutions (Prof A.Vendrik). In1964 I visited a summer research institute of the New York university in Saranac Lake, and worked on experimental emphysema in rats. After finishing medical training and military service, I started in the department of physiology of the university of Nijmegen in 1973 (Prof.F.Keuzer). The subject was control of breathing in humans and anesthetized cats. Human pathophysiology of breathing was studied in patients with a hyperventilation syndrome, and in newborns with near sudden infant death syndrome. The latter was also studied in an experimental animal model in a ten-year collaboration with the university of Bochum (Germany) Prof. H.Loeschke and Prof. M.Schlaefke. I wrote a thesis on neurophysiology of the control of breathing in the animal studies, together with a bio-electric engineer (Dr.F.Smolders). I worked at the University of Bristol UK for some time in 1979-1980 (Prof.M.Purves) on peripheral chemoreceptors and measuring respiratory oscillations in blood gas values. In 1983 I was invited to head the lung function department of the department of pulmonology . Since I had got familiarized with several basic techniques and ideas about sleep and breathing, it was not very difficult to start recording respiration in sleeping COPD-patients, and I performed a number of studies for various therapies for hypoventilating COPD-patients. Obstructive sleep apnea could not be ignored. This resulted in the first sleep laboratory in a pulmonology department in the Netherlands. Apart from that, exercise physiology, pulmonary function measurements by general practitioners, and pharmacological stimulation of breathing in COPD-patients, pulmonary diseases and work, were important topics of research. I was (co-) author in almost 300 papers in various journals, I had 35 Ph-D students, and finally, two years before retirement, was awarded in Princeton USA for my work on the hyperventilation syndrome. I retired at the end of 2004.

HISTORY

Being the eldest son of a General Practitioner, I studied medicine in Nijmegen, intending to follow my father and eventually take over his practice. This was not to be. In the 3rd year of

my medical studies (1963), I apparently performed well in an exam on medical physics, and got invited to work for three months in the department of medical physics on the in vivo measurement of oxygen saturation in the solutions with cell-free hemoglobin. Eventually, I stayed for three years. This was so challenging and interesting, that it made me decide to give up my ideas of becoming a general practitioner. Physiological research was to be my future. The principle of oximetry was known by that time, but only in-vitro, in a sample of hemoglobin in a cuvette, in a spectro-photometer. In-vivo measurements of oxygen saturation in intact humans, which we now take for granted was unheard of. At that time I also met Ton Coenen for the first time; he was working in the same Department of medical physics

Medical physics is a profession of physicists, working on problems in medicine. Rather complicated for a medical student and a future doctor. Realising this, I contacted prof F.Kreuzer in the department of physiolology, enquiring about possibilities for working in his lab after finishing my medical studies. His positive response resulted in a visit to a summer research institute in Saranac Lake (NY) in the USA. I participated in a project on a pathophysiological model of emphysema in rats. These animals were made to inhale an aerosol of a surfactant. The idea was, to disturb the normal physiological surfactant, and to wash this surfactant away from the animal's lungs. This was broncho-alveolar lavage 'avant la lettre'. The lungs were excised after one week. The compliance of these isolated lungs were measured, and tissue slides were made to study the ratio of tissue versus air in the treated and untreated lungs, in order to quantify possible emphysema. The results were not staggering, but the process of thinking about making a pathophysiological model, about ways of measurements for testing one's hypothesis, process data with statistics (in the pre-computer era) working in an international group of students, using a foreign language for communicating about science and for every-day needs, travelling abroad and finding one's way, was invaluable for my later work.

After graduating from medical school and after military service, I started as a Ph D student in the department of physiology in Nijmegen. At that time, one of the major questions in control of breathing was the (mathematical) description of ventilatory responses to increased levels of CO2 in the arterial blood (hypercapnia) en to lowered levels of O2 in the blood (hypoxia), as well as the responses to combined hypoxia and hypercapnia. Dr Smolders, bioelectric engineer, Dr Bernards, physiologist, and myself started to work on this matter in awake humans. Automated systems to change blood gas levels in a closed spirometric system were build. Volunteers, i.e. co-workers, medical students, family and friends were recruited to be subjected to these experiments. Ethical committees did not exist in those days, so every experiment that could be conceived, could also be realized in an extremely short time. I always made a point of being the first human subject in any experiment I ever did on human subjects. The experiments in intact awake humans did give good overall descriptions and mathematical equations about the behavior of the 'black box' of control of breathing. In order to get an insight in the subsystem of this black box of the overall response, we had to perform experiments on anesthetized cats that could be operated upon, and thus opened possibilities to study subsystems within the control system of breathing. For instance: can the neural activity in the efferent phrenic nerve be used as a measure of minute ventilation in anesthetized and paralysed cats? How do respiratory neurons in the brain-stem react to hypoxia, hypercapnia and asphyxia? How do the medullary respiratory neurons generate an increase in ventilation when chemoreceptors are stimulated? What is the effect of selectively eliminating either the central chemoreceptors at the ventral surface of the medulla oblongata, or the peripheral chemoreceptors in the carotid and aortic bodies? The techniques for these studies were operations on anesthetized, paralysed and ventilated cats, where one was surgeon, anesthesiologist, and neurophysiologist at the same time. Recording electrical

activity from the phrenic nerve or from single respiratory neurons in the brain stem was greatly supported by Frans Smolders, the biomedical engineer whom I worked with for many years, and who taught me about input impedance of amplifiers, about microelectrodes with tips of one micron for recording single unit activity of respiratory neurons, about quantifying the electrical output of the respiratory centres, and about mathematical models and curve fittings. This might have been the period of my life where I learned the most in the shortest time, about things that I did not learn in my medical education. Dr Smolders and I together wrote one thesis, with different responsibilities for different chapters, and defended this thesis in 1976.

After the steady state studies, i.e. give some CO2 to breathe and wait until the response of the ventilatory system is completely stable after 5 or more minutes, we were also interested in the dynamics of the responses of the ventilatory system. Generate a step-change in CO2, and measure the time constant of the ventilatory system i.e. the speed of the response. What made the ventilatory response as slow as 3 to 6 minutes: was it the chemoreceptors, the respiratory centres , the blood-brain barrier, the response of the cerebral blood flow? All these subsystems had to be isolated and measured separately in anesthetized cats, in order to understand the speed of response of the total system.

The sudden infant death syndrome (SIDS) was described around 1975. Newborn infants, usually less than one year old, who were apparently completely healthy, were found dead in their cots. One child was admitted to the department of neonatology in Nijmegen, as a so-called near-miss SIDS. Suspecting a failure in respiratory drive during sleep in this infant, we measured the ventilatory response to hypercapnia . This was not an easy thing to do in a newborn. We were struggling with the basic physical law that one cannot take measurements from a physical system without influencing this system at the same time. Applying a face mask to an infant would undoubtedly have a profound effect on the sleep-stage of the child, and consequently also on breathing. A wakefulness drive would stimulate and maintain breathing during such an intervention. No instruments that would measure ventilation and end-tidal CO2 were commercially available. Thus we had to build our own instruments. A three centimeter long accordion-shaped balloon as used in bicycle brakes was connected to a pressure monitor, and strapped around the thorax with a white shoelace. This was our respiratory movement monitor. A small tube (inner diameter 0.5 millimeter) was stuck under the nose with adhesive plaster, and a sample of expiratory air was led to a capnograph adapted to small flow rates of 50 ml/min. We applied a CO2-enriched air-mixture in a small oxygen-tent. It appeared that this child's ventilation hardly responded to an increase in end-tidal CO2. Thus, it seemed that there was something seriously wrong with the chemoreceptors of this child. Consequently, its nocturnal breathing was monitored (with an alarm system for apnea) at home. In spite of this monitoring, the child succumbed one month later. Autopsy revealed that structures of the external arcuate nucleus at the ventral surface of the medulla were absent. These structures are at the location of the central chemoreceptors for CO2! At that time this problem was sometimes called: 'Ondine's curse syndrome'. Ondine was a waternymph in ancient German mythology. Her lover Hans was not so monogamous as she would have liked. So, she cursed him that he would have to stay awake in order to keep breathing. If he would fall asleep, breathing would stop, and Hans would die. It was also claimed that Solomon's judgment on the two mothers, one of them having lost a child during the night, was the first description of the Sudden Infant Death syndrome.

In cooperation with the University of Bochum in Germany (Prof. Schlaefke) we developed an animal model in cats for this SIDS-problem. We were able to coagulate the central chemoreceptors in cats in a chronic model. When these cats fell asleep, they either strongly hypoventilated or showed apneas.

In 1983, I changed jobs from the department of physiology at the University of Nijmegen, to the department of pulmonology and became head of the lung function department. Breathing during sleep hardly was a scientific topic at that time. There was an early paper of Von Bülow, mainly descriptive about breathing and sleep (about 1950's). There was important work from John Remmers at Dartmouth medical college USA in sleeping dogs. He stated that during slow wave sleep, ventilation was driven by chemoreceptors. Ventilatory responses to CO2 were intact during slow wave sleep. However, during REM-sleep, these ventilatory responses were virtually absent. Also in awake subjects that were reading aloud from a book, he found no ventilatory responses to CO2. He claimed that the REM sleep breathing was driven by 'behavioral mechanisms' as in the awake reading subjects. John Stradling in Oxford UK found divergent respiratory phenomena during sleep and disagreed with Remmers. In Australia, Collin Sullivan (1981) hosted his more or less overweight mother-in-law. He observerd that this woman often fell asleep during the day, and often stopped breathing and snored considerably during these naps. She seemed to make respiratory efforts, but no air came out of nose or mouth. Obstructive sleep apnea was born. Collin Sullivan also started the first continuous positive airway pressure (CPAP) treatment on his own mother-in-law, and succeeded.

The problem of obstructive sleep apnea gradually became wider known and was recognized more often. Having had experience in measuring breathing during sleep in newborn, it was not really difficult to set up facilities for doing the same in sleeping adults. The things that one would like to measure in these patients were: Airflow at the nose and mouth, oxygen saturation in the arterial blood, respiratory movements of the chest, EEG and EOG, eye-movements, heart rate, and leg movements (restless legs syndrome).

As inspiratory air has a temperature of 20 degrees and expiratory air is 37 degrees, one only needs a small fast responding thermometer, in order to qualitatively measure airflow at nose and mouth. Thermistors could be bought easily and when soldered to a battery and a writing recorder, gave a nice and easy instrument to assess oronasal airflow, in a qualitative way. Some investigators later claimed that this could be a way to diagnose nocturnal hypoventilation. As the temperature changes due to breathing could never exceed the ranges from room-air to body temperature, these thermistors could by definition never be quantitative. So, hypoventilation (which would become important in diagnosing nocturnal breathing in patients with COPD) could never be diagnosed with thermistors only. Oxygen saturation was initially measured with an ear-oximeter developed by Hewlett and Packard. A bulky instrument with even a bulkier earlobe sensor measuring 12 by 12 by 3 cm, connected to the amplifier with a finger-thick cable with optic fibres. Again, one cannot measure from a physical system without disturbing the system: patients slept badly with such an oximeter strapped to their heads. From the start, heart rate was also provided by the oximeter. Thoracic movements were measured with the pneumatic transducer made from little balloons from bicycle brakes, and strapped around the thorax. Respiratory inductance plethysmography (respitrace) was to come on the market only at the end of the 1980's. EOG and EMG was measured with second-hand monochannel ECG-amplifiers, and recorded directly on a writing paper recorder. End tidal CO2 was sampled with a narrow tube taped on the upper lip, and sampling from one nostril. No CO2 signal meant apnea. A rise in end-tidal CO2 was the ultimate proof of nocturnal hypoventilation. Video recording was made when other problems were suspected such as restless legs syndrome. Patients did not like this video recording; they felt their privacy was invaded too much.

Studying obstructive sleep apnea syndrome (OSAS) was not our primary interest. It was only a byproduct from our main interest: sleep and hypoventilation in patients with COPD. However, as we had the means to study OSAS as well, this was a nice way to be able to help many non-COPD-patients. The therapies for OSAS were abundant: weight reduction, tennis

balls in the back of the pyjama, snore-alarm with electric stimuli , tongue retainers, CPAP and an Ear-Nose-Throat (ENT)-surgical procedure: uvulo-palato-pharyngo plasty (UPPP), and sclerosing the soft palate with microwave needles. Initially these therapies were not very well validated. Therefore, the Rotterdam ENT group (Prof F.van der Meché) started a multicentre trial around 1992, paid for by the health care insurance providers ('ontwikkelingsgeneeskunde'), to evaluate CPAP versus UPPP therapies. The Nijmegen group consisted of my department and of the ENT-department (Prof. H. Manni). Seventy-five patients were randomized for each treatment. Before the data were eventually processed and published, it had already become clear in the literature, that CPAP was the first choice therapy for OSAS.

At that time there was also a discussion about who's territory was this OSAS problem. Directly connected was the problem for general practitioners about the referral pattern for their patients. Neurologists and neurophysiologists traditionally were the group of doctors who knew about sleeping problems. Pulmonologists were the doctors with expertise on breathing. ENT specialists were the experts on the problems of large tonsils, large tongue, narrow oropharynx etc. Vested interests of specialist doctors in the various hospitals also were a component in this 'battle for OSAS-patients'. Many hospitals were able to form a multi disciplinary team of Pulmonologists, ENT specialists and neurologists, but in several hospitals there was a severe competition between the various specialists.

As stated above, in a chest-clinic, our main interest was in nocturnal hypoventilation in COPD patients, especially in the category of the 'blue and bloating' type. The Netherland's Asthma Foundation (NAF) provided a grant for a study on the prevalence of this problem, predictive value of daytime measurements of ventilatory responses to CO2, daytime blood gas values, and therapeutic possibilities of stimulating nocturnal ventilation with the acidifying diuretic acetazolamide or with the progestative hormone chlormadionacetate. Dr. P. Vos wrote an outstanding thesis on this subject (1992). Both drugs did stimulate nocturnal breathing, resulting in a decrease in nocturnal PaCO2 by approximately 0.5 kPa, and nocturnal oxygen saturation increased by 4% in patients with COPD. The progestative chlormedadionacetate was somewhat less effective than the diuretic acetazolamide.

The respiratory muscles of COPD patients are weakened and dysfunctional because of the continuous state of hyperinflation and continuous contraction of the diaphragm. This means that these patients use their intercostals muscles as main breathing muscles. During sleep, especially during REM sleep, the intercostals muscles are subject to REM sleep paralysis, leaving the diaphragm as the only respiratory muscles to sustain ventilation. This will lead to hypoventilation and nocturnal hypoxemia in these patients. We reasoned that respiratory muscle training might improve the function of this diaphragm, and thus alleviate this nocturnal hypoxemia in COPD. Dr. Y. Heydra studied this hypothesis and reported in her thesis in 1995. A new way of respiratory muscle training was devised: Target flow inspiratory muscle training. A 10-week training program resulted in an increase in diaphragmatic strength by 3 kPa, and a mean improvement of nocturnal oxygen saturation of 1.9%.

Some patients with COPD hypoventilate and become hypoxemic during day and night. They are called the blue and bloating type of COPD. Others fight and struggle to maintain the levels of oxygen and CO2 in their blood at normal levels. They are called the pink and puffing type of COPD. The severity of the COPD, as expressed by the degree of airway obstruction does not explain this difference in the two subtypes of COPD patients. The level of CO2 in the body is measured by the peripheral chemoreceptors in the carotid bodies and by the central chemoreceptors at the ventral surface of the brainstem. Actually, these central chemoreceptors measure CO2 in the cerebrospinal fluid. The CO2 is also controlled by the autoregulation of the cerebral blood flow. The higher the CO2 in the cerebro-spinal fluid, the

greater the cerebral blood flow to carry the excess of CO2, away from the brain. We hypothesized that het blue and bloating COPD patients, that are hypoxic and hypercapnic during the day but especially during the night, might have an overactive autoregulation of the cerebral blood flow. This problem was studied by Dr. M. van de Ven who wrote her thesis in 2001. She was able to measure cerebral blood flow, and its reaction to changes in CO2, noninvasively in intact human subjects, using the near infra-red spectroscopy method. COPD patients did have a lower cerebral blood flow, and a lower reaction of the blood flow to increases in arterial CO2. However, no differences were found between the normocapnic COPD patients and the hypercapnic ones.

COPD patients often need treatment with diuretics, because of edema and overfilling of the circulation. The first drug of choice is the loop-diuretic furosemide. However, this drug causes a metabolic alkalosis and therefore, it can be expected that this drug will cause a greater or lesser degree of ventilatory depression. Dr F.Brijker studied this problem in a group of COPD patients who were on furosemide (2001). After inclusion, they were randomised either to continue with the furosemide, or receive placebo for one week. After that week, the drug regimen was changed again, in a double blind fashion. During the placebo treatment, the arterial CO2 decreased significantly by 0.5 kPa. Mean nocturnal oxygen saturation did not change significantly. Consequently, it was concluded that furosemide depresses ventilation in COPD patients.

Chemical stimulation of ventilation by either inducing a metabolic acidosis with the diuretic acetazolamide, or by giving progesterone acetate, was revisited in a study by Dr. M. Wagenaar. He elaborated on the underlying mechanisms of these drugs, in a study on cats and on humans. This study was supported again by the Netherland's Asthma Foundation, and was performed in close cooperation with the Department of Physiology of the University of Leiden (Dr.L.Teppema). The study focused on stimulating the central or peripheral chemoreceptors by both drugs. The methods used, were developed in Leiden: the dynamic end-tidal forcing technique. Not only were both drugs studied separately, but also in a combined treatment regimen (hoping for a positive interaction between the drugs). Especially the nocturnal PCO2 values benefited from the combined therapy of two weeks: a decrease of 1.4 kPa. The nocturnal oxygen saturation increased during combined therapy by 4.7%. The effects of the combined therapy were significantly better than both drugs alone. The cat-experiments showed that there was a true interaction between the drugs: the effect of the combined therapy was more than the added effects of both single therapies. Dr. Wagenaar defended his thesis in 2003.

Around 1995, Henk Hassink pulmonologist in Venlo and myself, started a working group "Sleep and Breathing" within the Dutch Pulmonologist's association: NVALT. From the start, this group was open to other specialists. Exchange of information, reviewing the literature, exchanging clinical experiences, relationships with health care insurances and with commercial firms was discussed in twice-yearly meetings in the various clinics. I was to preside this working group for about 5 years. In 2004 we wrote a standard guide on nocturnal oxygen supplementation in COPD-patients with nocturnal hypoventilation and hypoxemia. It was not clear whether such patients, being hypoxemic only during the night, were at risk for developing pulmonary hypertension. Careful scanning of a wide range of literature (Dr. K.Orbon) provided no evidence of pulmonary hypertension in this category of patients. Consequently, the relevant clinical conclusion was that COPD patients with isolated nocturnal hypoxemia did not need treatment with nocturnal oxygen supplementation via a nasal canula or face mask.

The early members, or 'founding fathers' of the working group 'Sleep and breathing' were pulmonologists, ENT-specialists, neurologists, oro-facial surgeons of the clinics of

Nijmegen, Amsterdam, Heeze, Venlo, Arnhem, Groningen, Leeuwarden and Rotterdam. At later stages many more clinics from all over the country joined.
In 2001, I was to head an expertise centre for Work and Pulmonary disease. The minister of health had provided funding for four of such expertise centres for work and: lungs, skin, musculo-skeletal system, and psychological system, in order to reduce sick-leave and to reduce disability due to diseases of these organs. One could apply and write a business plan for such a centre. The Nijmegen business proposal for a centre for work and pulmonary diseases ended on the first place. My directors asked me to head this centre, as I also had written the business plan. I did so, until my retirement in 2004. Therefore the Nijmegen sleep centre was to be headed by someone else: my first PhDstudent Dr.P.Vos with whom I had worked on sleep and breathing, for almost 15 years.

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SLEEP-WAKE
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PhD Theses

MAPPING INSOMNIA
BRAIN STRUCTURE, FUNCTION AND SLEEP INTERVENTION

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THE RESEARCH PROJECT

A sleepless night (sleep deprivation) may hamper task execution of tasks requiring concentration, attention and working memory the next day. Such problems were thus far not found unequivocally in people who reported chronic sleep problems. In this thesis we investigated if and in what way cognitive performance (memory, attention and working memory tasks) and brain activity are different between people with chronic sleep problems (chronic insomnia) and normal sleepers. We also investigated whether any deviations in behavior and brain activity as a result of insomnia normalize after sleep therapy without medication. Lastly, we tried to find a better model than total sleep deprivation in good sleepers to investigate deviations in cognitive performance and brain activity in insomnia. For this we applied a method that makes the sleep more shallow but does not change total sleep duration.

We limited our study to older adults, as insomnia mainly occurs in people over 50 years of age. Sleep disorders as a result of (other) physical or mental disorders (so-called secondary insomnia) as well as sleeping disorders resulting from respiratory problems (sleep apnea) or restless legs during nighttime were further excluded. Any results could thus be directly related to sleep problems, and confounding factors such as breathing difficulties or mental problems could be excluded.

Subjects were selected by means of sleep registration, among other methods. During nighttime, an electro-encephalogram (EEG) was administered, leg movements were measured by means of electrodes and possible breathing difficulties were detected through a flow meter and an oxygen saturation meter. In addition, questionnaires were administered. From over four-hundred candidates a group of older adults was selected who only suffered from chronic sleep problems - primary insomnia. We compared this group to a group of normal sleepers, who we also subjected to sleep measurements and questionnaires to exclude sleep disorders, mental problems and other factors that might bias the study.

Both groups underwent brain scans that measured brain activity during task performance (functional magnetic resonance imaging, fMRI). By means of double-pulse transcranial magnetic stimulation (TMS) we attempted to gain an insight in the balance between the excitatory (stimulating) and inhibitory (repressing) brain cells in the neuronal network of the cortex. The participants were also asked to perform computer tasks that measured, amongst others, the level of attention (vigilance).

A second, identical session took place after six weeks of sleep therapy (for half of the group of insomnia patients) or after a similar time interval without sleep therapy (for the other half of the group of insomnia patients). This sleep therapy consisted of a combination of methods known to be effective for treating insomnia. The time that patients could spend in bed was for instance limited (sleep restriction), patients were exposed to 30 minutes of bright light every morning and evening to regulate melatonin production (light therapy), and they took a hot bath two to three hours before going to bed alternated with fairly intensive bodily

exercise (body temperature regulation). The intake of coffee and alcohol was reduced and activities allowed in bed were restricted; e.g. they were not allowed to eat or watch TV in bed (sleep hygiene).
To find a better model than total sleep deprivation for the deviations in cognitive performance and brain activity in insomnia we applied a method that induces shallow sleep, but does not affect total sleep duration, to a group of persons without sleep complaints. We achieved this by suppressing the slow brain waves that are characteristic of sleep (slow wave suppression). During one of the two sessions, participants were exposed to beeping noises which started as they entered deep sleep and which volume increased with continuous deep sleep; the beeping noises only stopped when the subjects' EEG indicated that they entered more shallow sleep. Cognitive performance and brain activity were measured the next day to evaluate whether the effects of this intervention showed a similar profile to that of the insomnia group.

SUMMARY OF THE INDIVIDUAL CHAPTERS

Chapter 1 provides an overview of the scope of the thesis and summarizes what is known thus far about the cognitive performance and brain activity in insomnia and after sleep deprivation, and what the treatment of insomnia involves. One third of all adults, in particular older adults, complain of suffering from insomnia. Although task performance is not affected in laboratory settings, insomnia is related to an increased number of industrial accidents and absenteeism from work. Insomnia is characterized by a state of elevated "arousal" (hyperarousal), which is shown from different physiological measures and which can be detected in the brain through increased brain activity before sleep onset and during sleep. Several factors can be distinguished that (1) make someone vulnerable for insomnia, (2) could lead to insomnia and (3) maintain insomnia. An increased sensitivity to the activating function of stress hormones could play a role in the onset and maintenance of insomnia. Insomnia is also known to be related to problems with emotion regulation: emotions are internalized instead of expressed or acted upon. Non-pharmacological sleep therapy may, particularly in the long term, be more effective than sleep medication for the treatment of insomnia.

Chapter 2 further elucidates the specific research questions featured in the various chapters.

Chapter 3 describes the performance of insomnia patients on two attention tasks. In one task, participants were required to press a button as soon as an asterisk appeared on a computer screen (the simple vigilance task). Intervals between asterisk appearances varied. Duration of the task was approximately 12 minutes. In the second, more complex task, participants were asked to press a button as soon as they saw the letter 'p' but not when the letter 'd' appeared (the complex vigilance task). Here, too, the intervals were variable and task duration was about 12 minutes. Compared to normal sleepers, insomnia patients were faster on the simple, but slower on the complex vigilance task. After sleep therapy this effect was restored; they became slower on the simple, but faster on the more complex task. This may be explained by the 'hyperarousal' effect, which influences the reaction time as long as the task involved is simple, but does not last on a more complex task which requires decision-making abilities. Sleep therapy may 'normalize' the insomnia patients so that their performance is similar to that of normal sleepers.

Chapter 4 describes the performance of both groups on a task that involved generating as many words as possible; in a particular category, for instance animals (categorical fluency

task) or generating words starting with a particular letter (letter fluency task). Insomnia patients show decreased brain activity in the frontal parts of the brain that are normally activated during this task (the lower winding of the frontal cortex -the inferior frontal gyrus) and the more centrally located frontal cortex (medial prefrontal cortex). After sleep therapy the brain activity in these regions partly recovers. Even on baseline, insomnia patients score better on both tasks than normal sleepers, possibly due to hyperarousal. Sleep therapy even improves their performance, particularly on the more complex task, the letter fluency task. It is possible that here, too, hyperarousal plays a role.

Chapter 5 describes how insomnia patients have lower grey matter density in three brain regions, namely the orbitofrontal cortex (in the frontal part of the brain, above the eye socket), and two regions in the precuneus (located in a higher, rear and more central part of the brain). Grey matter density in the orbitofrontal cortex has a strong correlation with insomnia severity: the more a patient suffers from insomnia, the lower the density. This area is often active during decision making. The precuneus is involved in a network of areas activated when the brain is at rest, the so-called 'default network'. It may be important in future research to evaluate whether insomnia patients show less strong brain waves during wakefulness and sleep.

Chapter 6 describes a double-pulse transcranial magnetic stimulation (TMS) experiment. With this method it is possible to measure the occurrence of inhibition or facilitation of the evoked muscle response, the so-called motor evoked potential (MEP), when a stimulus with a variable interval is preceded by a prior and weaker stimulus. Compared to normal sleepers the insomnia patients showed a strong MEP after a double pulse, but also after a single-pulse stimulus. This led to a relatively decreased facilitation effect compared to normal sleepers. Sleep therapy did not normalize this effect. The results suggest that, here too, hyperarousal may play a role.

Chapter 7 describes how slow wave suppression of normal sleepers may influence performance on attention tasks. Slow wave suppression led to lapses, moments of total lack of attention, in which no responses are generated. These lapses occurred during the simple as well as during the complex attention tasks. The performance after slow wave suppression very much resembles the performance after total sleep deprivation, as described by others, but does not resemble the performance of insomnia patients as described in chapter 3.

Chapter 8 discusses the influence of slow wave suppression on memory performance and brain activity. After slow wave suppression, the activity of the hippocampus, a brain area important for memory, was less strong than after a normal night of sleep. Participants could also remember less well which pictures they had or had not seen previously.

In **Chapter 9** results are summarized and discussed in the context of what is known about insomnia thus far. Cognitive performance in insomnia seems particularly affected in tasks with longer duration which involve a decision-making process. Even when task performance is not affected,
brain activity of insomnia patients may be different from that of normal sleepers; sleep therapy may partly reverse this. Grey matter differences are found in different regions than the regions where functional differences are found between insomnia patients and normal sleepers. The regions where grey matter differences are found are strongly correlated to functions found to be affected in sleep deprivation and insomnia, such as decision-making. The involvement of these areas in the occurrence of spontaneous oscillations (brain waves)

during rest are an indication of the importance of mapping the default network in insomnia. The irreversibility of deviant intracortical facilitation in insomnia patients further suggests a possible risk factor for developing insomnia. Studies mapping the long-term effects of sleep therapy are required to determine which structural and functional differences are reversible and which are not. We did not find support for our hypothesis that slow wave suppression in normal sleepers might offer a more valid experimental model for insomnia than total sleep deprivation.

On the basis of these results a model for the development of insomnia is proposed in which combinations of (perhaps unalterable) factors, such as genetic factors, with secondary factors, such as stressful events, might lead to insomnia and/or psychiatric complaints. An unalterable factor could be a factor that makes a person vulnerable to develop a particular condition. Such factor could be grey matter density differences or a difference in sensitivity for intracortical facilitation. Secondary factors, such as a traumatic event, would then determine whether the vulnerability factor develops into a disorder or not. Here, too, longitudinal studies can offer a better insight into the tenability of this model.

The current results give reason to detect and start treatment for insomnia earlier in the future. Improvement is possible at many levels: from a more frequent referral of the general physician to sleep clinics to availability of non-pharmacological sleep therapy and reimbursement of this treatment by health insurances. Future longitudinal research could shed more light on the reversible and non-reversible effects of insomnia. More research is also needed to investigate the relationship between insomnia, decision-making skills and emotion regulation; suggestions for questionnaires and tasks are given. Setting up a large-scale registry of people with unexplained sleep problems whose symptoms are further mapped by measuring performance and brain activity may result in a definition of the various subtypes of primary insomnia. This could lead to quicker recognition and treatment of this frequently occurring, limiting but treatable condition.

Commentary on the dissertation by Ellemarije Altena

MAPPING INSOMNIA

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Sleep is commonly considered to exert a life-sustaining function maintaining the integrity of brain and body physiology. Still, the prevalence of insomnia in the general population raises the issue of how these patients sustain chronic sleep disruption and restriction, and how insomnia impacts on cognitive performance and even alter the underlying brain structure and function.

To address these issues, Dr Ellemarije Altena resorted to modern techniques of neuroimaging to probe brain structure and function in carefully selected populations of insomniac patients. She first analyzed structural brain scans in insomnia patients and normal controls. She showed that patients had a lower grey matter density in the orbito-frontal areas, involved in decision making and the evaluation of affective stimuli.

She used functional MRI to assess the neural correlates of executive functions in insomniacs. She shows that, relative to normal matched controls without sleep complaints, the insomniacs recruit frontal areas to a lesser extent during a verbal fluency task. These reduced responses were observed despite a superior performance of insomniac patients. Even more intriguingly, sleep therapy resulted in a partial recovery of these functional abnormalities.

She also used double pulse transcranial magnetic stimulation and showed that intracortical facilitation is reduced in insomnia patients, possibly due to a higher baseline response.

Finally, in order to investigate the consequences of poor sleep in normal volunteers without sleep complaints, she evaluated the effects of slow wave sleep suppression by auditory stimuli. In collaboration with Dr Van Der Werf, she was able to show that slow wave sleep suppression was associate with a deficit in hippocampal responses during subsequent encoding of novel pictures and their recognition was impaired during later retest.

These studies pave the way for the detailed assessment of the structural, functional and cognitive aftermath of chronic insomnia and open new avenues to better understand both the mechanisms of insomnia and its detrimental consequences on brain function. The main challenge of future research will be to try to tease apart causes from consequences, genetic from environmental factors, as well as the relations between insomnia and psychiatric disorders.

SLEEP-WAKE
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Mini review

HISTORY OF ELECTROENCEPHALOGRAPHY

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PROLOGUE

An important year in the history of electroencephalography was 1890. In that year an article appeared in the authoritative scientific journal 'Centralblatt für Physiologie' about the recording of electrical waves from the brains of animals [1]. The paper was written by the Polish neurophysiologist Adolf Beck, a young assistant affiliated with the Department of Physiology of the Jagiellonian University of Kraków (Poland), guided by the famous physiologist Napoleon Cybulski. It was a short summary of research which Beck had performed on the electrical brain activity of rabbits and dogs. Following this attention-drawing publication in the Centralblatt, a violent polemic arose between brain physiologists in this journal. The issue concerned the claim about the discovery of electrical brain activity.

RICHARD CATON (1842-1926)

The discussion was abruptly ended by a letter from Professor Richard Caton (Figure 1) of the School of Medicine at Liverpool (UK) [2], who referred to a brief abstract of about 10 sentences, published 15 years earlier in 1875 [3]. In this abstract, which appeared on the occasion of a meeting of the British Medical Association in February 1875, Caton described the spontaneous waxing and waning of the electrical waves recorded from the brains of rabbits and monkeys. Caton expressed his findings as following: 'In every brain of monkey or rabbit hitherto examined the galvanometer has indicated the existence of electric currents. The external surface of the gray matter is usually positive in relation to the surface of a section through it. Feeble currents of varying direction pass through the multiplier when the electrodes are placed on two points of the external surface, or one electrode on the gray matter and one on the surface of the skull. The electric currents of the gray matter appear to have a relation to its function'. In a longer paper published two years later in 1877 [4], Caton more extensively described identical experiments with a larger number of animals with generally the same results. Caton's claim was convincing and indisputable and nowadays it is generally accepted that his abstract from 1875 contains the first description of the electroencephalogram.

Richard Caton was an Edinburgh graduate in 1867. A year later he settled in Liverpool, and became physician and lecturer in physiology at the Royal Infirmary School of Medicine in Liverpool. Caton was inspired by du Bois-Reymond's book 'Untersuchungen über thierische Elektricität' and started with work on the animal brain, using non-polarisable electrodes and a string galvanometer. After defending his priority in having made the discovery of electrical brain waves, he did no further work on the brain. For many years his family and colleagues were unaware of his discovery. This was possible partly because of many other things that he did in his long life, but also because he took deliberate steps to hide the fact that he had worked on the brain of animals. The most important of these other activities was a study of the treatment of rheumatic heart disease. The basis of his treatment, complete bed rest , is still recommended today. Caton's interest in his university never waned, and he reached the

high office of pro-chancellor. Later, he became a city councillor and devoted much time to the promotion of public health. In 1907 he was elected Lord Mayor of Liverpool and, as such, became more well-known than as a brain scientist. This does not exclude the fact that Richard Caton is presently recognized as the discoverer of electrical brain waves, which form the basis of electroencephalography.

Figure 1. Richard Caton, shown in his thirties when he was working on electrophysiology.

MORE DISCOVERY CLAIMS

Before Caton finished the discussion in the Centralblatt, some submitted letters gained extra attention. Physiology professor Ernst Fleischl von Marxow from the University of Vienna wrote that he had already, seven years earlier, deposited a covert letter at the Imperial Academy of Sciences in Vienna containing claims about electrical brain activity [5]. Indeed, in this sealed letter, indications of electrical brain activity based on experiments carried out in Vienna were made, but his observation missed crucial points. The response to Fleischl's paper was rather laconically: 'Nature held and still holds innumerable riddles under the seal of secrecy. It makes no difference for science whether these riddles are kept secret under the seal of Nature herself or under that of the Imperial Academy of Sciences in Vienna' [6]. A second response about Beck's paper in the Centralblatt of 1890 came from Francis Gotch and Victor Horsley [7]. Although they referred to papers slightly related to the subject, it was notable that they mentioned the electrical response to sensory stimulation. Gotch, a direct colleague of Caton and the descriptor of the refractory phase that takes place between nerve impulses, performed experiments showing the electrical responses of the mammalian spinal cord to cortical stimulation. He did this together with his brother in law, the famous Horsley, the designer of the stereotactic apparatus for brain research. Just as Fleischl, however, Gotch and Horsley overlooked essential elements, such as the spontaneous oscillations and the cessation of these fluctuations after stimulation.

The most interesting response, however, came from Wassili Y. Danilewsky (Figure 2), a scientist working at the University of Charkow (Russia, now Ukraine) [8]. Danilewsky studied at the University of Kazan in Russia, together with Wladimir Ulyanov, later known as Lenin, and he finished his study at the University of Charkow in 1877. His doctoral thesis dating from that year was entitled, 'Investigations into the physiology of the brain', and was written in Russian. In his letter to the Centralblatt, Danilewsky mentioned his non-published doctoral

thesis. Indeed in this manuscript a description of the spontaneously fluctuating brain potentials of a dog's brain could be found and also indications for the changes in brain potentials after stimulation. Unfortunately, Danilewsky published a summary of his thesis in his response-publication to Beck not earlier than 1891. Nevertheless, what remains after the melee of claims to the Centralblatt is that it is almost certain that Danilewsky was the first scientist after Caton to observe electrical potentials of the brain.

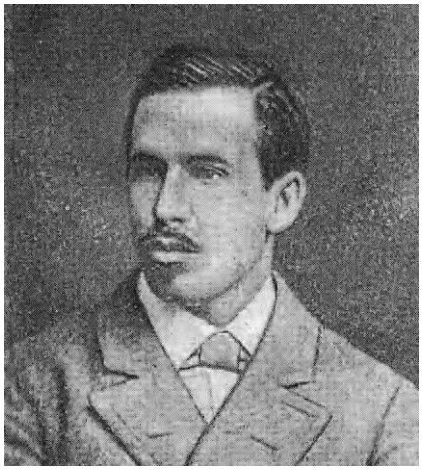


Figure 2. Wassili Yakovlevich Danilewsky (1852-1939) in 1879, shortly after he finished his thesis.

ADOLF BECK (1863-1942)

Beck was not aware of Caton's work, but he explored electrical brain activity, together with his supervisor Napoleon Cybulski (Figure 3), much more extensively and detailed than did Caton. Beck delivered important contributions to the nature of electrical brain activity. He accurately described the localization of sensory modalities on the cerebral cortex by electrical and sensory stimulation and by recording the electrical activities with clay electrodes and a string galvanometer. The abstract he sent to the Centralblatt in 1890 was a summary of his extensive thesis which was published one year later in the Polish language [9], and later, at the initiative of the expert in the history of neuroscience, Mary Brazier, was translated into English [10]. Beck explored, in frogs as well as in paralyzed dogs and rabbits, the parts of the cortex that reacted upon stimulation with electronegativity. This was done for several sensory modalities and in fact this was the first description of 'evoked potentials'. In doing this, Beck also found the spontaneous oscillations of brain potentials and showed that these fluctuations were not related to heart and breathing rhythms. Moreover, Beck brought up a new element: the potential decrease upon sensory stimulation. He observed a cessation in the fluctuations of the electrical waves as a consequence of afferent stimulation, either by electrical stimulation of the nervus ischiadicus or by peripheral stimulation of the eyes with light flashes. Thus, he was the first to describe the desynchronization in the EEG following stimulation [11, 12]. For all his work Beck was thrice nominated for the Nobel Prize, but never received that high honour.

In 1895 Beck left Kraków and moved to the University of Lwów, at that time a sister university of the Jagiellonian University in Kraków. Nowadays, Lwów, previously called Lemberg, is a city in the west of the Ukraine, known under the native name Lwiw. At an age of 32, Beck was appointed professor in physiology and founded, in Lwów, the Department of

Physiology under the Medical Faculty. Beck continued research on the nervous system, but also started research in general physiology and wrote a textbook on human physiology together with Cybulski. During 1904 and 1905, Beck served the Faculty as Dean and in 1912 he was nominated as Rector of the university and again in 1913. During World War I, Beck was a prisoner of the Russian army and was released with the help of Pavlov. Beck retired in 1932. In World War II, Lwów was occupied by the Nazis, who started to imprison all Jewish inhabitants. Beck was of Jewish origin. To avoid ending up in the gas chamber of the Janowska concentration camp, Adolf Beck committed suicide, with poison he got from his son Henryk, a medical doctor, who shortly after the war died in Breslau (Wrocław) in 1946. The last sentences that Beck's daughter Jadwiga, whose husband Kazimierz Zakrzewski was executed by the Nazi's in 1941, wrote about her father were: 'His death was painfully tragic: in 1942 in Lwów, when this magnificent, strong man had reached the age of 80, after a beautiful and dedicated life, he took poison when the Germans came for him' [13]. Born on 1 January 1863 in Kraków, Beck died on an unknown day in August 1942 in Lwów.

Figure 2. Adolf Beck (left) and Napoleon Cybulski (right), writing the textbook 'Fizjologia człowieka' ('Human physiology') in 1915.

FIRST ELECTROENCEPHALOGRAPHIC REGISTRATIONS

Wladimir Wladimirovich Práwdicz-Neminski (also called Nemminski) was the researcher who in 1913 published the first photographs of electrical brain potentials of dogs [14]. He did this with a mirror galvanometer and could register the movements of a beam of light on photographic paper (Figure 3). Before that time, researchers were not able to register electrical activity on paper. They were just looking at the moving coil of the galvanometer. Beck, for example, measured the size of the deflections by visual observations with a millimetre scale, and noted them by hand (on paper) as fast as possible.

Práwdicz-Neminski who worked at the Ukrainian Academy of Sciences in Kiev was the first who could distinguish two different rhythms in the electrical brain waves of dogs. Initially, he denoted these rhythms as 'waves of the first order' and 'waves of the second order' (Práwdicz-Neminski, 1913). These waves were later called A waves and B waves, and nowadays respectively alpha and beta waves. It was also Práwdicz-Neminski who coined the German term 'Elektrocerebrogramm' for the electrical brain activity [15].

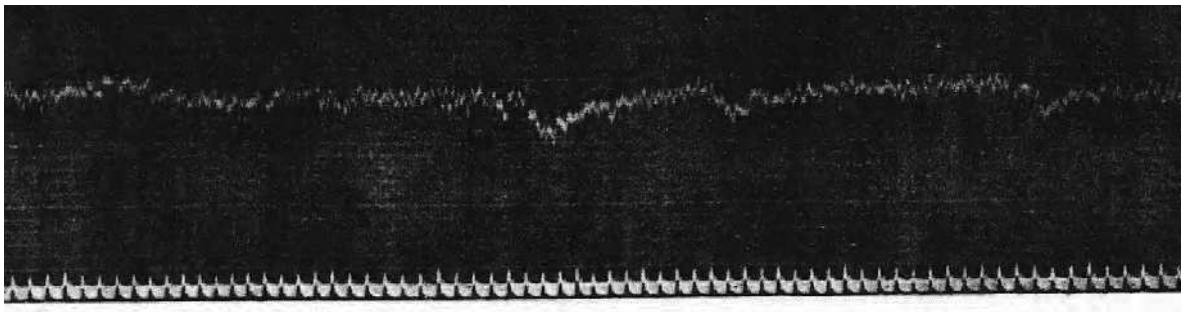


Figure 3. The first registration of the electrical brain activity (Elektrocerebrogramm) of dogs made by Nemminski in 1913 [14]. The lower trace is the time in seconds (5 units is 1 sec.). The recording has a dominant frequency of 12 to 14 Hz.

HANS BERGER (1873-1941)

In 1929, almost forty years after Beck, Hans Berger published his first paper about recordings of electrical activity from the surface of the human brain [16]. His children, especially son Klaus and daughter Ilse, were obedient, but also often unwilling subjects. On 14 October 1927, Berger exclaimed: 'Eureka! The waves of Klaus are identical to the intra-cerebral recorded waves. I am able to record the electroencephalogram of an intact skull!' (Figure 4). Berger was the first to record the electrical activity of the human brain and so promoted the technique into being a non-invasive one. Moreover, it appeared from his first publication in 1929, that Berger was well aware of studies published earlier. In an interesting historical introduction in his lengthy paper, he gave full credit to all researchers who had already described main phenomena, such as the spontaneous fluctuations, the blocking after sensory stimulation and the existence of two pattern rhythms [16]. Moreover, Berger described the conditions under which the two rhythms, alpha and beta rhythm, appeared in humans. After having registered the changes in the electrical wave pattern during sleep and narcosis, and the aberrant activities during epileptic attacks [17], Berger came to the conclusion that the discovery of the EEG was not only a major breakthrough in neurophysiology, but also that this technology was of outstanding importance for its diagnostic value. Finally, Berger changed the term 'Elektrocerebrogramm' into 'Elektrenkephalogramm', which for linguistic reasons in English became 'electroencephalogram', abbreviated to EEG.

Born in 1873 in the town of Neuses, near Coburg, in the south of Germany, Berger earned a doctorate of medicine at the University of Jena in 1897. In 1901 he became lecturer and staff member in psychiatry and neurology at the Psychiatric Clinic of this university. Berger was interested in the relation of brain activity and 'psychic' activity. His original view was that he could measure this psychic activity with the electroencephalogram, but early on, around 1910, gave up this idea and pursued another line of inquiry, taking a more pragmatic approach. Berger became interested in clinical applications of electrical brain activity, and he reactivated his brain studies by making recordings of humans. In 1919 Berger succeeded Otto Ludwig Binswanger as Chairman of the Psychiatric Clinic of the University. With his strict nature, however, every day he found time from 5 to 8 pm to continue his experiments, even in the academic year 1927-1928 when he served his university as Rector. Berger had a more sensitive double-coil galvanometer than his predecessors, while more powerful amplifiers came on the scene. In 1924 he was already able to record the electrical brain activity in patients with skull defects in an intracerebral way, but it wasn't until 1927 that he could

make recordings directly from the intact skull. Berger faced an analogous problem as Beck: the ‘waves of the first order’ (the A or alpha waves) appearing under low active brain states, were larger than the ‘waves of the second order', (the B or beta waves), arising under higher mental activities (Figure 5). Since high frequencies waves were masked due to the galvanometer slowness, this counterintuitive finding was not understood for a long time.

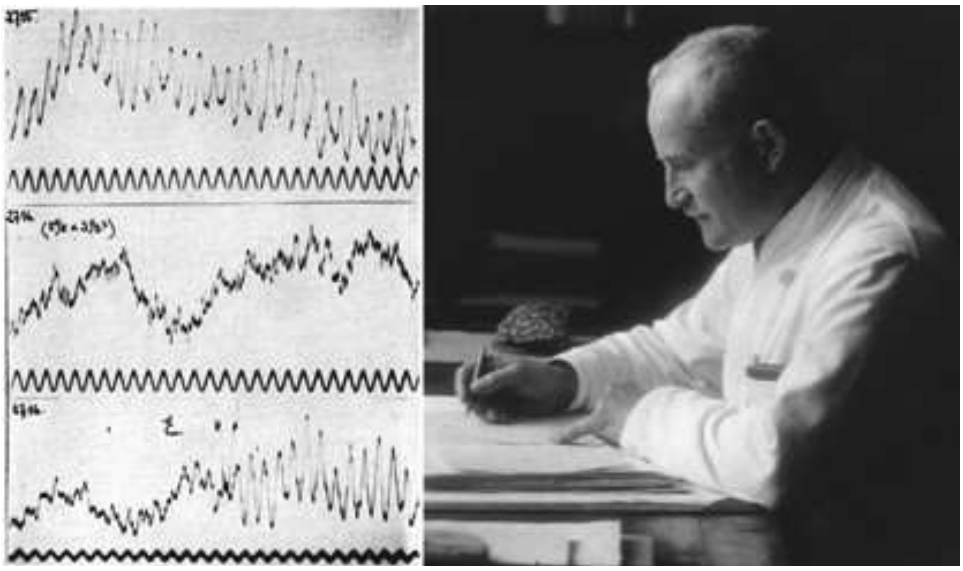
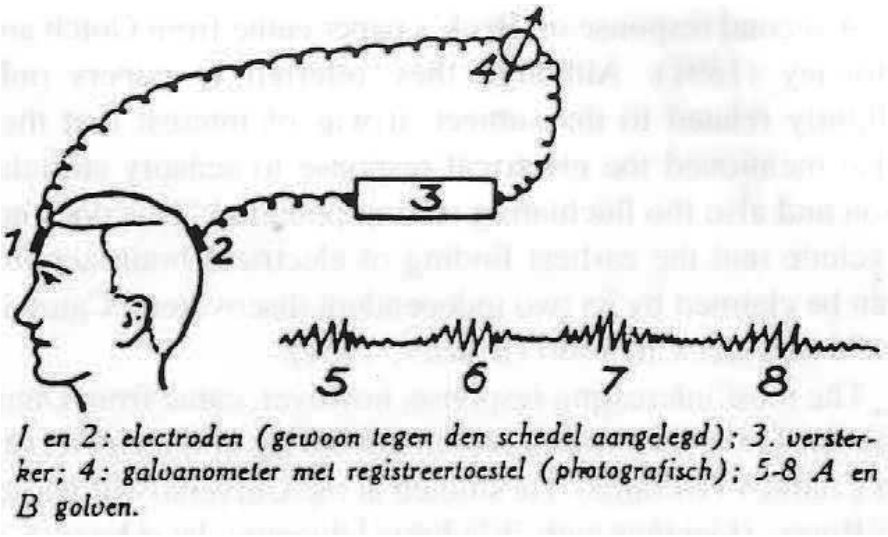


Figure 4. Hans Berger in 1927, with the ‘Elektrenkephalogramm’ of his daughter Ilse. Upper trace: Ilse in rest (alpha waves), middle trace: in calculating a sum (beta waves), and lower trace: in giving the outcome of the sum (mixed waves).

The important findings of Berger were largely ignored and neglected by the scientific community, but his international reputation was slowly growing. This brought the modest Berger to the International Congress in Psychology in Paris in 1938, where he was almost recognized as a celebrity. Back in Germany he found, however, only humiliation especially by the Nazi regime, who distrusted his work. The Nazis also forced him to give up his Chair at the Psychiatry Clinic in 1938 and closed down his laboratory. They even did not allow him to receive the Nobel Prize in Stockholm for which he was nominated. Berger fell into a severe and long depression and on 1st of June 1941 he took his life by hanging. Berger’s wife, Freiin Ursula von Bülow, had a hard time, also since son Klaus fell on the battlefield in Russia half a year later. Many similarities can be seen in life and work of the two great pioneers of electroencephalography: Adolf Beck and Hans Berger.

ACCEPTANCE OF THE ELECTROENCEPHALOGRAM

The intensive disputes in the Centralblatt starting in 1890 made clear that the question of electrical brain activity was a lively issue of various researchers in Western, Central and Eastern Europe, including Russia and the Ukraine. It also accentuated the main role which researchers from Eastern Europe played at that time in this scientific field. The leading role for these scientists in neurophysiology was, however, abruptly ended by the Soviet regime, that made a choice for the Pavlovian concept, better fitting into the ideas of this dogmatic regime. This overshadowed all neurophysiology, which as a field of research, quickly disappeared.



1 en 2: electroden (gewoon tegen den schedel aangelegd); 3. versterker; 4: galvanometer met registreertoestel (photografisch); 5-8 A en B golven.

Figure 5. The first drawing in a Dutch newspaper ('De Haarlemsche Courant', from 1930) referring to the measurement of electrical brain activity. A and B waves are visible and desynchronizations are shown. The recording is from Berger's son Klaus [16].

Until the Thirties, the value of electrical brain waves was not appreciated and this explains why many researchers ignored publications appearing before and during Berger's work. This kind of research came too early; the time was not yet ripe to see the importance of the curious electrical activity directly measured from the skull, very different from the well-known action potentials from nerve cells and axons. An additional reason might be that the majority of reports were published in languages not well accessible to a large scientific audience. Nevertheless, an assistant of the American neurophysiologist Hallowell Davis noticed Berger's 1929 paper in 1933 by accident. Davis could not believe that the rhythmical alpha waves originated from the brain and considered this strange and global electrical activity as an artefact. His colleagues Edgar Douglas Adrian and Bryan Matthews of the University of Cambridge (UK) were also sceptical, but these recognized neurophysiologists working on action potentials from large nerves, started in 1934 to replicate the recordings of the obscure German psychiatrist [18]. Lord Adrian appeared to have a beautiful alpha rhythm and was so fair to propose this rhythm being named as the 'Berger rhythm', but this honour was too much for the reserved Berger.

EPILOGUE

After repeated replications, Berger's results were taken seriously, and he was able to convince people of the value of the new method. Many scientists, such as the Gibbs couple, Wilder Penfield, Herbert Jasper, Frédéric Bremer and Nathaniel Kleitman, began to use the electroencephalographic technique, mainly in epilepsy and sleep-wake research [19]. Since that time this oldest brain imaging method became a valued tool in fundamental and clinical neurophysiology, and was even later introduced in cognitive research. The first international conference on electroencephalography was held in London in 1947, while the journal 'Electroencephalography and clinical Neurophysiology' ('the EEG journal') was founded in 1949. For the reason that Hans Berger published the first human recordings of the electroencephalogram and made this tool indispensable in neurophysiological and psychophysiological research, he is now considered as the father of electroencephalography.

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SLEEP-WAKE
Research in The Netherlands

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Research papers

R278995/CRA0450, A NOVEL SELECTIVE CRF1 RECEPTOR ANTAGONIST MODUATES REM SLEEP IN RATS: RELEVANCE FOR DEPRESSION

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INTRODUCTION

A marked deregulation of the hypothalamic-pituitary-adrenal (HPA) axis activity is found in depressed patients and other psychiatric diseases such as acute and chronic stress. Direct evidences relate abnormalities of the HPA axis functions with polysomnographic alterations in patients with depressive illness, in which sleep disturbances were reversed after corticosteroid replacement therapy. A decreased responsiveness to dexamethasone has been repeatedly found in depressed patients, it is considered as evidence for an increased activation in the pituitary on ACTH and cortisol secretion. The increased cortisol secretion was correlated to shortening of rapid eye movement (REM) sleep latency in patients[1-2]. Evidence from animal studies showed a reduction in waking and an increase of sleep time in a rat strain that synthesize low levels of the corticotropin-releasing factor (CRF), or following administration of the CRF antagonists (α-hCRF) and astressin, or antisense of the CRF mRNA[3]. The CRF, a 41-amino acid peptide is one of the hypothalamic mediators, which can play a critical role in the proper functioning of the stress response by acting through two related CRF receptor subtypes CRFr1, CRFr2. The CRFr1 is widespread in the brain, whereas the expression of CRFr2 variants is limited to subcortical structures. The implication of the CRFr1 in the pathophysiology of depression and/or anxiety has been postulated. Additionally, the selective CRFr1 antagonist R121919 has been shown to effectively attenuate stress and normalize the sleep pattern in a rat line selectively bred for high innate anxiety[4].

In the present study, we sought to further characterise in rats the profile of the selective CRFr1 antagonist R278995/CRA0450 on sleep-wake EEG behaviour. This compound displays also high affinity for the σ1 receptor, and shows anxiolytic and antidepressant-like activities in rodents[5].

METHODS

Animals and Surgery

Male adult Wistar rats (n=16), weighing 240-260g at the time of surgery and during recovery were individually housed in full-view Plexiglas cages (30 cm diameter, 40 cm high) and maintained in sound-attenuated room and controlled environmental conditions throughout the study: 22 ± 2°C ambient temperature, the relative humidity at 60%, light-dark cycle (lights on from 6:30 to 18:30; light intensity ~100 lux). The animals have free access to standard laboratory food chow and tap water.

Surgery was carried out under deep anaesthesia (Pentobarbital 10 mg/kg i.p. and Thalamonal, 1ml s.c.). Two electrodes were placed stereotactically on each side of the sagittal suture for the recording of the frontal and parietal cortical electroencephalogram (EEG) (AP + 2 mm, L 2 mm, and AP – 6 mm, L 3 mm from Bregma, respectively). Stainless

steel wire electrodes were subcutaneously implanted on each side of the orbit and in the dorsal neck muscle recording of for monitoring the electro-oculogram (EOG) and the electro-myogram (EMG), respectively.

EEG recording, Pharmacological treatment and Sleep-wake organisation analysis

The animals were placed into the registration box and the acquisition of EEG, EOG and EMG signals was performed on-line through a bipolar recorder amplifier (Embla-Somnologica, Flaga Medical Devices, Iceland), using a sampling rate of 100 Hz. Two EEG recordings of 8 hours were performed in 16 animals following the first oral administration of vehicle (0.3 % Tween 80/saline solution), and the second was performed for the same duration following 3 and 10 mg/kg (n=8 each group).

Post-acquisition analysis of sleep-wake parameters were done per 2h periods and were compared to control values. The EEG signals were scored in 15 seconds epoch and classified as being either wake (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2), rapid eye movement sleep (REMS). Duration of vigilance states, latencies and other sleep-wake parameters were calculated.

EEG power spectral analysis

Spectral analysis of the EEG was performed separately during W and SWS2 in each period of 2-hours of the recording session. The EEG signal digitised at a sampling rate of 100 Hz with 16-bit resolution and artefact-free 15 sec epochs were analysed off-line by Fast Fourier Transformation and power spectra was in five different frequency bands: 0.4 to 4 Hz (δ), 4.2 to 8 Hz (θ), 8.2 to 12 Hz (α), 12.2 to 14 (σ) and 14.2 to 30 Hz (β).

Statistical analysis

Sleep-wake parameters were expressed in minutes (mean ± S.E.M.). Power spectra were expressed as a percentage of control values in each frequency band separately during waking and deep sleep. A Wilcoxon sign-rank test was applied to assess significant differences values between drug and vehicle treatment.

RESULTS

The major changes in sleep behaviour were found during the first 2 hours following the oral administration of R278995/CRA0450 at 3 mg/kg i.e. a decrease of the amount of time spent in waking (-24%, $p<0.01$), an increase in time spent in deep sleep (+30%) and REM sleep (+118%, $p<0.05$). The time spent in light sleep was not

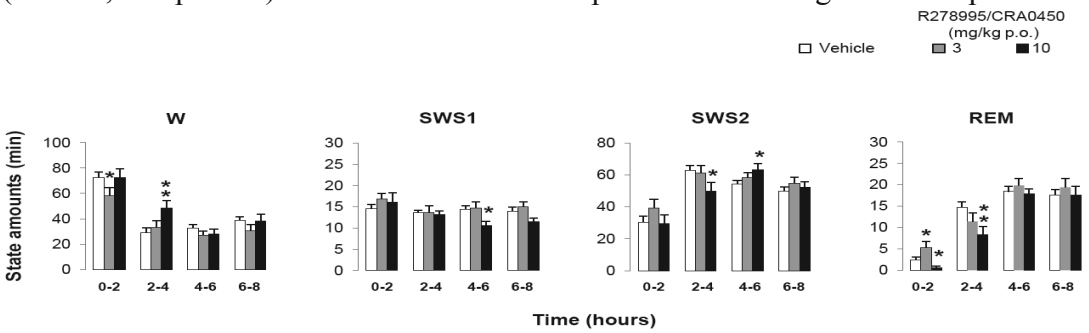

Figure 1: Effects of oral administration of R278995/CRA0450 (3 and 10 mg/kg) and vehicle on sleep-wake states during the four consecutive 2-h periods in rats. Data are mean values ± S.E.M. expressed in minutes for the states of wake (W), light slow wave sleep (SWS1), deep slow wave sleep (SWS2), and rapid eye movement sleep (REMS). * $p < 0.05$; ** $p < 0.01$: Wilcoxon signed rank-test versus baseline values.

modified. R278995/CRA0450 at the dose of 10 mg/kg increased the amount of time spent in wakefulness was during the subsequent 4 first hours of the recording session (+32%, p<0.01) relative to vehicle group. This increase was mirrored by a reduction of time spent in both deep sleep and REM sleep (–21%, -40%, p< 0.01). A decrease in time spent in light sleep was observed during the last 4 hours of the recording session (-25%, p<0.05) (Figure 1).
Examination of sleep latencies indicated that the R278995/CRA0450 lengthened specifically the latency of REM sleep onset (+64%, p<0.01).

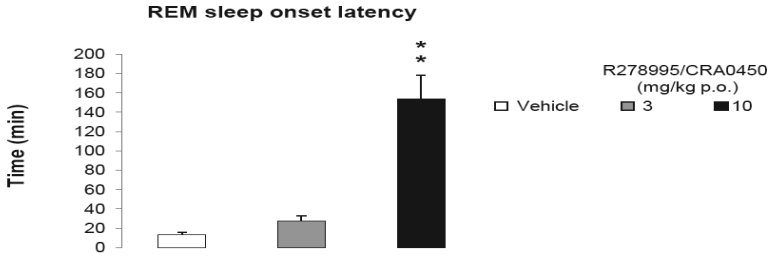

Figure 2: Effects of oral administration of R278995/CRA0450 (3 and 10 mg/kg) and vehicle on REM sleep onset latency. Data are mean values ± S.E.M. expressed in minutes. ** p < 0.01 Wilcoxon signed rank-test versus baseline values.

R278995/CRA0450 at 3 mg/kg had no major effects on the sleep-EEG spectra during wakefulness; however a significant reduction in the power over all frequency bands was observed during deep sleep (Figure 3). Separate analysis demonstrated no consistent effect of R278995/CRA0450 at 10 mg/kg on power spectra in both vigilance states.

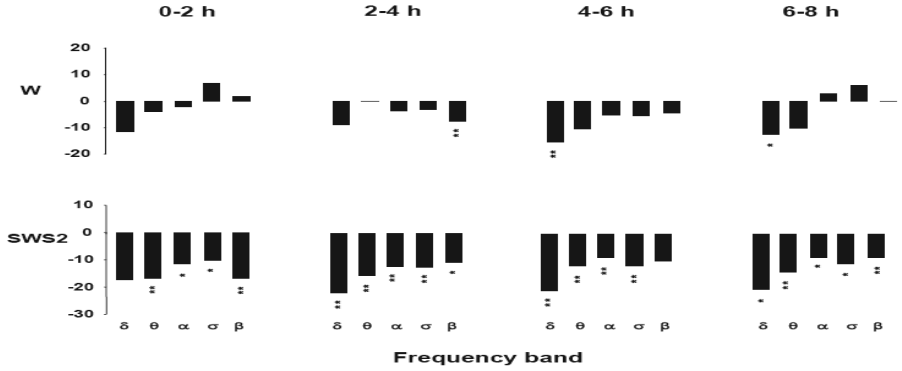

Figure 3: Changes in the EEG power spectral activity expressed in percent of baseline during wake (W) and deep slow sleep (SWS2) during four consecutive 2-h periods after oral administration of R278995 (3 mg/kg) in rats. * p < 0.05, ** p < 0.01, Wilcoxon signed rank-test versus baseline values.

DISCUSSION

In the present study, R278995/CRA0450 at lower dose reduced time of wakefulness and enhances deep slow wave sleep while decreased EEG power spectra in different frequency bands. At the higher dose, the compound produced a clear reduction in time spent in REM sleep and lengthening of REM sleep onset latency without any consistent effect on power spectra in both vigilance states. The current data, in particular at the lowest dose of CRF1r antagonist, are in agreement with earlier reports showing that either central or peripheral administration of specific CRF antagonists and CRF antisense DNA oligodeoxynucleotides in non-stressed rats reduced spontaneous wakefulness, however no conclusions were drawn in these studies regarding the effects on REM sleep stage[3-6]. The current findings in rat

demonstrate an important step in differentiating the contribution of CRFr1 in the modulation of REM sleep. Interestingly, these data are in line with antidepressant-like activity of R278995/CRA0450 at higher dose observed in animal models of depression such as learned helplessness and the olfactory bulbectomy models in the rat[5].

A possible action of orally application of R278995/CRA0450 includes the locus coeruleus nucleus, the dorsal raphe (DR) and the laterodorsal or pedunculopontine tegmentum neurons located in the mesopontine area of the brainstem. Immunohistochemical and CRFr1 biding studies demonstrated the presence of CRF positive neurons in these brain nuclei involved in the control of sleep-waking cycle, emotion and cognition[7]. In addition, it has been shown that accumulation of extracellular 5-HT in the DR and forebrain area was imputable to CRF-induced increase in the firing activity of 5-HT neurons in the DR and mesolimbocortical serotoninergic circuits[8]. This prolonged 5-HT release and neurons excitability may potentiate GABAergic inhibition by 5-HT, and contribute to disturbance in the cellular mechanism involved in the regulation of sleep as short latency to REM sleep, high density of REM as well as frequent awakening observed in sleep pattern of depressed patient. This hypothesis is further supported by the fact that agents that facilitate GABAergic neurotransmission are typically anxiolytic, while agents that inhibit GABAergic neurotransmission are anxiogenic. Therefore, our findings may suggest that the antidepressant like profile of R278995/CRA0450 observed in sleep architecture in rats and in behavioural studies[5] could account in part for its effects in reducing the serotonin effects on GABAergic neurons transmission that are mainly affected following elevated CRF level to stress response.

R278995/CRA0450 also displayed potent antagonistic properties at σ1 receptor[5]. Recent evidence indicated an implication of σ1 receptor in neuropsychiatry disorders including anxiety and depression[9]. On one hand several clinically used antidepressants such as specific serotonin reuptake inhibitors fluoxetine and fluvoxamine as well as the monoamine reuptake inhibitor imipramine have been shown to bind this receptor with high affinity[10]. On the other hand, σ1 ligands enhance the firing of serotoninergic neurons in the DR[11], increase dopamine neuronal activity[12], enhance NMDA-induced noradrenaline release from the rat hippocampus slices[13] and acetylcholine from rat frontal cortex[14]. Therefore, we suggest that the antidepressant and anxiolytic-like activities observed in earlier behavioural studies are likely associated with the potency of the compound to block the CRFr1 and in part to its σ1 receptor component.

From a translational perspective it should be noted that sleep disturbances are commonly found in depressed patients i.e. disruption of sleep continuity resulting in reduced of total sleep time, lengthening of sleep onset, deficit of deep sleep, a great density of REM sleep with an earlier onset of this sleep stage and shift of REM sleep occurrence towards the beginning of the night. Interestingly, an association between CRF1 receptor and sleep disturbances has been reported in depressed patients[15]. In this later study, the administration of CRFr1 antagonist R121919 improved sleep, increased the amount of SWS and deceased REM sleep density in patients with major depression. Moreover, in preclinical studies R121919 showed high potency to attenuate stress-induced sleep alterations in rats with high innate anxiety behaviour[4]. Our present findings in this sleep rodent model showed a robust effect of R278995/CRA0450 on REM sleep variables i.e. significant reduction in REM sleep, lengthening of REM sleep latency, which fits the hypothetical role of CRFr1 antagonism antidepressant activity.

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SLEEP DEPRIVATION BY GENTLE HANDLING IN RATS DOES NOT AFFECT THE PHYSIOLGICAL, BEHAVIORAL AND HORMONAL RESPONSES TO NOVELTY EXPOSURE

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INTRODUCTION

Even though the exact function of sleep is still unknown, it is common knowledge that sleep loss is detrimental to good health. The underlying mechanisms are however still poorly understood, but several hypotheses have been formulated. One of the hypotheses is that some of the proposed detrimental health consequences of sleep loss are mediated by changes in the activity of the two major stress systems; the hypothalamic-pituitary-adrenal (HPA) axis and the sympathico-adrenomedullary (SAM) system. Activation of these systems results in the release of the glucocorticoid corticosterone and the catecholamines adrenalin and noradrenalin, which are known to have many different effects. Existing evidence supports the hypothesis that sleep loss activates these systems and that the released stress hormones are responsible for some of the detrimental health consequences (for review see[1]). Moreover, sleep deprivation (SD) may not only affect the basal activity of the stress systems, but can also affect the reactivity of these systems to other stressors and/or challenges[1-4]. Yet, few studies examined how sleep loss affects the activated stress response. This study aimed to assess how short-term, 4-h gentle handling sleep deprivation (SD), affects the physiological (heart rate and body temperature), behavioral (locomotor activity) and hormonal (corticosterone) responses to novelty exposure in rats. Novelty exposure has a long history in rodent stress research and is generally considered to be a minor stressor. It is however unknown whether minor stress responses are affected by sleep deprivation. This study also aimed to take measurements in a way that would minimize possible confounding stress effects of the sampling and of the sleep deprivation procedure.

METHODS

Animals and surgery

Male Sprague-Dawley rats (Harlan, The Netherlands), weighing 310-330 g at the time of surgery were used as subjects. The animals were individually housed under 12 : 12 h light/dark regime with a 30 min dim/rise period. Food and water were available ad *libitum*. Rats were implanted with a biopotential transmitter (DataSciences International) to record heart rate, body temperature and locomotor activity. In addition, a dual tubing jugular vein catheter was implanted. During the two weeks of recovery, the animals were daily handled and habituated to the sampling procedures. During the subsequent experimental period, potentially disturbing stimuli were carefully avoided.

Set-up and measurements

Recovery and adaptation started immediately following surgery when animals were placed in the experimental set-up. This 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 of the sampling procedures. It consisted of a telemetry set-up (DataSciences Int.) for continuous and automatic measurement of physiological and behavioral parameters using the implanted transmitter. Concomitantly, blood was sampled automatically and repeatedly through the jugular vein catheter using an automated blood sampling system (AccuSampler® Micro; DiLab®). Blood samples were stored at -80°C until assayed for corticosterone by radioimmunoassay (125I RIA Kit, MP Biomedicals, LLC).

Sleep deprivation and novelty exposure

A total of 8 animals were subjected to either SD or control conditions, and either with or without exposure to novelty, using a cross-over Williams design. This resulted in the following conditions: control + no novelty (n=8); SD + no novelty (n=6); control + novelty (n=6); SD + novelty (n=8). SD was induced during the first 4h of the light phase (resting phase) by using gentle handling[5]. Whenever the animal showed behavioral signs of sleep, it was stroked on its back or moved and it was occasionally handled briefly. This method has shown to effectively induce wakefulness for at least 80% of the SD period[5]. After 1.5 h recovery, the animals were exposed to novelty for 15-min according to the protocol described by de Boer and co-workers[6]. The experimenter entered the room and gently picked the rat up from its home cage and placed it individually in a new, clean, empty cage. After 15 min, the rats were returned to their home cages and a 2-h recovery period followed. Under control conditions, animals were left undisturbed in their home cages in the same experimental room.

Statistical analyses

Statistical significance was evaluated using the repeated measures ANOVA with treatment and time as factor variables, and the Greenhouse-Geisser correction for homogeneity in variance. *Post-hoc t*-tests were performed to determine the time points at which the groups differed. The statistics are expressed together for the same treatments (either SD or control) during the SD and 1.5 recovery period, and also together for the same treatments (exposure to novelty or not) during the 15-min novelty exposure and 2-h recovery period, since no significant differences between these conditions were found. All tests were performed at a significance level of 0.05.

RESULTS

Under control conditions, heart rate and body temperature gradually declined at the start of the light period, whereas locomotor activity declined rapidly. Plasma corticosterone was not significantly affected by the lights-on stimulus. As compared to the control conditions, SD by gentle handling was associated with a significant increase in heart rate and body temperature during the last 1.5 h of the SD period. Locomotor activity levels were significantly increased throughout the SD period. Plasma corticosterone was only significantly increased during the first 5 min of the SD. In comparison to the last 30 min of the dark phase immediately preceding SD, the levels of heart rate, body temperature and locomotor activity were not increased. This suggests that the levels were maintained at the levels seen during the active phase. At termination of SD, levels of all variables immediately returned to undisturbed control values. During the SD and the 1.5 h period thereafter, there was no significant difference between the two SD and also not between the two control conditions. Exposing the control and sleep-deprived animals to novelty induced a fast increase in heart rate, a tendency for a delayed increase in body temperature, a fast increase in locomotor activity, and a delayed increase in plasma corticosterone as compared to animals not exposed to

novelty. After termination of novelty, the time courses of the responses varied but all reached control levels within 30-min. During the 15-min novelty exposure and the 2 h period thereafter, there were no significant differences between the two groups exposed to novelty and also not between the two groups not exposed to novelty. A small tendency for a faster recovery to novelty seems however visible for the sleep-deprived animals.

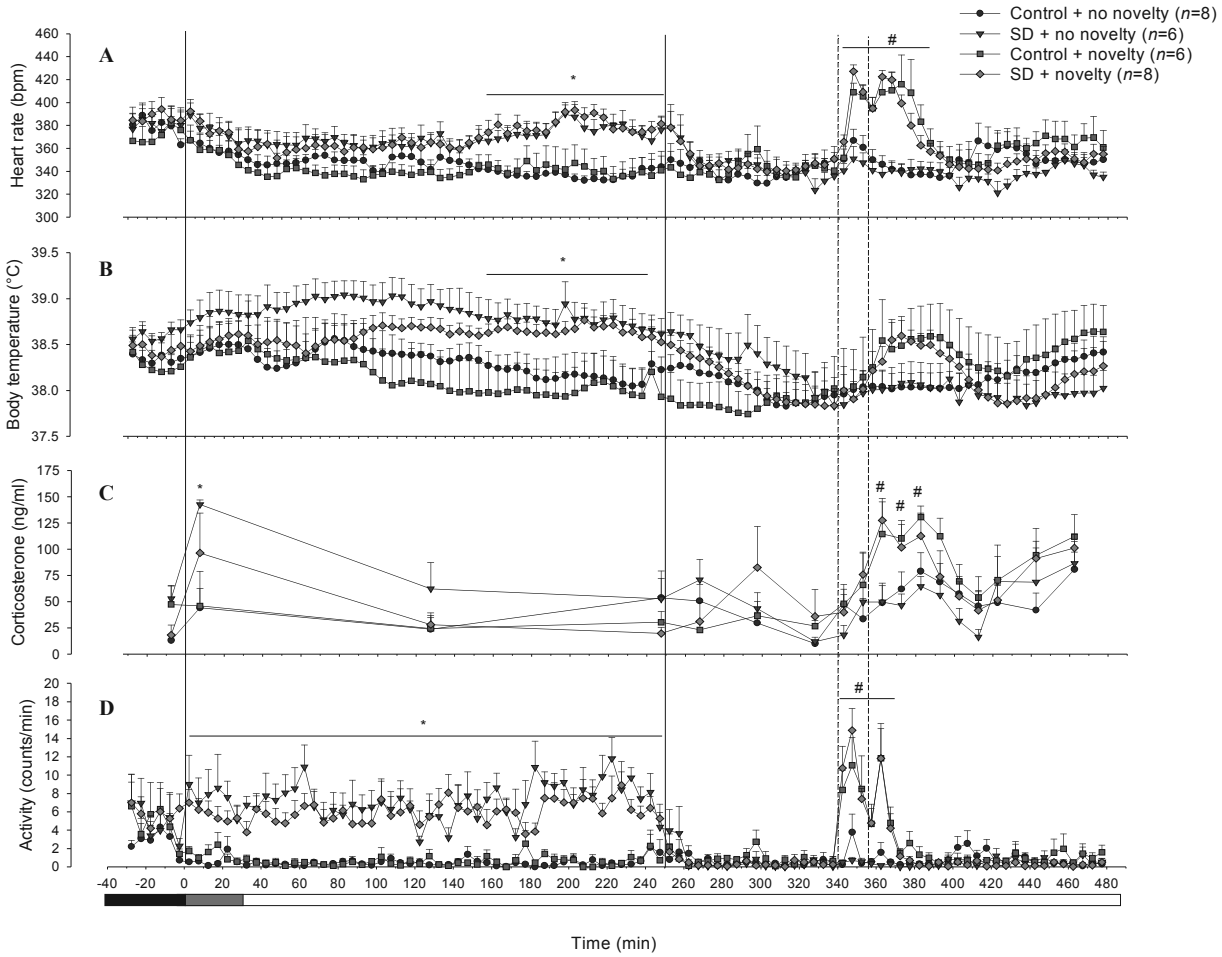

Figure 1. Time course of the changes in heart rate (**A**), body temperature (**B**), plasma corticosterone (**C**), and locomotor activity (**D**) for the animals exposed to 4-h SD and the control animals with or without exposure to novelty. Data are expressed as 5-min averages + SEM for the physiological measurements and for every 10-60 min + SEM for the corticosterone measurements. The responses are shown for the last 10 - 30 min of the dark period, the 4 h (+ 5 min) SD period (indicated by area between the solid lines) starting at lights-on followed by the 1.5 h post-SD period and the 15-min exposure to novelty (indicated by the area between the dashed lines), and the 2-h post-novelty period. The bars underneath the graph indicate the dark (black), the 30-min rise (gray) and light (white) period. *Type effect: SD versus control during SD and 1.5-h recovery period, $p<0.05$. #Type effect: novelty exposure versus no novelty exposure during 15-min novelty exposure and 2-h recovery period. All parameters showed significant time*treatment interaction.

DISCUSSION

Exposing animals to 4-h SD by gentle handling at the beginning of the light phase caused levels locomotor activity, heart rate and body temperature to stay above those of the undisturbed predominantly sleeping controls, but below levels as reached during the end of the dark period. Plasma corticosterone was only increased during the first 5-min of the SD and reached levels above those at the end of the dark period, but the levels were below those seen during mild stress[4,7]. Furthermore, stable control values were reached immediately upon termination of the SD. This shows that the HPA axis and SAM system were activated in response to 4-h gentle handling SD, but only mildly. The 15-min novelty exposure also induced activation of the stress systems as was shown by the increases in heart rate, body temperature, locomotor activity and plasma corticosterone. There were no significant differences in the responses to novelty between the control animals and the sleep-deprived animals. Thus, the reactivity of the stress systems to novelty was not affected. This is in agreement with other studies showing that changes in physiological stress reactivity may occur with chronic sleep restriction but not with acute, short-lasting SD (for review see[1]). The activation of the stress systems by SD and by novelty exposure was not affected by confounding stress factors of the applied sampling procedures. This is confirmed by the normal and stable measured physiological values together with the low plasma corticosterone levels under control conditions. The used SD method also minimized possible confounding stress effects, as is shown by the mild activation of the stress systems in response to the SD, which is mostly activity-related. Thus, stress does not appear to be a confounding factor in our method of gentle handling SD and in the used sampling methods. In conclusion, based on our measured parameters, 4-h SD by gentle handling does not affect the reactivity of the stress systems to novelty exposure. This indicates that rats exposed to acute SD can still deal adequately with a minor stressor.

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SLEEP FEATURES AND NIGHTLY BODY MOTILITY IN ELDERLY PEOPLE

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INTRODUCTION

Sleep architecture changes with age. Elderly sleep is characterized by an increased sleep latency, by a decreased total sleep time, caused by a reduction in slow wave sleep as well as in REM sleep, by more light sleep, and, in particular, by nighttime awakenings [1]. All these changes seem to indicate a weaker circadian regulation of the sleep-wake cycle in aged people, also when they are completely healthy. Nocturnal awakenings leading to sleep fragmentation, commonly not occurring in young people, are a typical characteristic of elderly sleep.
A question addressed in the present study is whether the fragmented sleep of elderly people is associated with a change in nightly motility. Gori and colleagues found in a polygraphic sleep study in elderly subjects a decrease of gross body movements, suggesting dissociation between sleep fragmentation and body motility [2]. A decrease in sleep position shifts in older people was also described by De Koninck and coworkers [3]. On the other hand, an increase in nocturnal activity, measured by actigraphy, was established in the heavily fragmented sleep of Parkinson patients [4]. Thus, the question remains what kinds of changes occur in sleep motility with ageing and how they are related to sleep fragmentation. To approach these issues in more detail, the motility structure of nocturnal sleep was investigated in elderly subjects. This was performed by measuring changes in body position in sleep by a body position meter and by establishing the overall nightly activity by actigraphic measurements.

METHODS

A total of 20 participants (8 men and 12 women) were recruited from two nursery institutions for elderly people in Amsterdam: Beth Shalom (6 subjects) and Amsta (14 subjects). The age range of people was 66 to 92 years, with a mean age of 83 years. Before participation, subjects got an intake in which their physical condition as well their sleep behavior was discussed. Exclusion criteria were sleep medication, misuse of drugs and alcohol, and psychiatric problems. All subjects declared themselves to be healthy. The subjects were informed that their sleep movements, in relation to the quality of sleep, should be monitored. Subjects signed an informed consent, under the condition that they could stop at any time with the experiment. After the complete study all participants received € 100.
Subjects were monitored for two successive nights in their home situation, being a room in the institution. Subjects went to bed and got up just to their own needs and wishes, on their own preferential times. Two couples slept in separate beds in the same room, and all other subjects slept alone. Before the start of the experiment subjects were provided with an actigraph (Actiwatch), worn around the non-dominant wrest, measuring all physical activity of the subjects, expressed in a number of counts. Subjects got also a belt around the chest containing a body position sensor (Pro-Tech Embla). Positions in bed, distinguishing supine, prone, and left and right lateral body positions, were measured continuously. Turnings in bed

could be easily determined in this way. After a shift in body position, thus a complete or half turn, no measurements were performed during the following two minutes.
Just after final awakening in the morning, the Amsterdam Sleep Quality Scale, with some additional questions, was filled in, in order to get a subjective impression of sleep [5]. Temperature in the sleeping room was measured continuously and was approximately 20 degrees Celsius. The sleep of the second night was analysed in order to avoid eventual first night effects. With a t-test (SPSS version 11.5) putative differences between the data of this experiment with data of a previous analogue experiment with the same design, but with young subjects (mean age 31 years with a range between 23 and 46 years), were established [6].

RESULTS AND DISCUSSION

Subjects of the present study with a mean age of 83 years (range between 66 and 92) go, on the average, to bed at 23.06 (range 20.34–0.40) and get up at 7.24 (range 6.36-8.12), thus having a mean total bed time of 8.18 hours. The subjectively estimated mean sleep time is 7.26 hours and the objectively mean sleep time of such a night, estimated by actigraphy, is 7.23 hours. Table 1 shows the data of sleep features of the analyzed night.

Sleep efficiency	73.8 ± 2.5	n = 17
Fragmentation index (%)	37.4 ± 5.2	n = 17
Sleep quality (scale 0-14)	8.6 ± 1.0	n = 20
Body position shifts	14.1 ± 2.0	n = 18
Nightly activity (counts x 1000)	13.5 ± 2.9	n = 17

Table 1. Data (mean and SEM) of the body position sensor (body position shifts) , the Actiwatch (nightly activity, sleep efficiency and fragmentation index) and the sleep quality scale. Due to incidental failures of body position sensor or actigraph not all n's are 20.

Sleep efficiency is approximately 74. In the literature, this is reported to be approximately 80 for a mean age of over 70 [1, 7]. This fits well for this age group, with a mean age above 80 years. In a previous study approximately 94 was found for young subjects, which is significantly higher ($p < 0.05$) [6]. Sleep fragmentation is considerable with aged people. The fragmentation index is approximately 37, while the normative score for middle aged people is about 15 [8]; a figure which is confirmed by a previous study [6]. Mean sleep quality is approximately 9, which is lower compared to young people with a score of 12 ($p < 0.05$) [6]. A score of 9 can be regarded at best as moderate sleep quality. Despite the objectively fairly low quality scores for their nocturnal sleep, subjects did not complain about their sleep.

The number of body shifts, determined by the body position sensor, was established for the whole night of the subjects, consisting of the time running between the subjectively indicated time of falling asleep till the subjectively indicated time of waking up in the next morning. The average for the investigated nights was a mean of approximately 14 body shifts per night. Compared to the number of body shifts per night for younger people [6], this points to a reduction in gross body movements in aged people. The conclusion that old people make less gross movements expressed in shifts in body position and turnings during sleep, is also

supported by literature data [2, 3]. This might be related to the lowered quality of elderly sleep with increased fragmentation, although the causal connection of movement reduction is still not completely clear.

All kinds of motor activity were determined by the Actiwatch during the entire night. The objectively indicated 'sleep start' and 'sleep stop' by the actigraph is fully comparable to the upper mentioned subjectively obtained start-stop data. Viewed against the perspective that gross movement, such as body position shifts and turnings, tend to decrease with age, it is remarkable to notice a higher degree in actigraphically determined motor activity in elderly people. Although the individual data show a high variance, the difference between old and young subjects is significant ($p<0.05$); approximately mean 13 500 counts per night for old subjects and approximately mean 4 000 counts per night for young people [6]. Obviously, elderly people show more small movements than young people. This counterintuitive finding can be explained by the process of a significantly increased fragmentation in aged subjects, with more periods of awakenings and arousals, by less deep sleep and by more light sleep, together with a lowering in sleep efficiency and quality. Movements commonly consist of small shifts of head and extremities with stretching or bending arms or legs, and all kinds of other small muscle movements. All these movements are not resulting in shifts in body position and in body turnings, and are thus not seen by the body position sensor. In Parkinson patients the same phenomena can be seen, even to a greater extend. Parkinson patients have motor disorders which are associated with painful postures and with problems with turnings in bed. In these patients, a decreased mobility is expected, but due to the serious sleep fragmentation together with the typical Parkinson tremors and movements, an increase in nocturnal counts and motility by actigraphic measures has been found [4]. Thus also in these patients a decrease in body shifts and turns is associated with an increase in small movements, and with a low sleep quality.
Obviously, nocturnal movements in elderly people split up in two categories: movements related to gross body position shifts and turnings, presumably related to physiological needs, and movements related to unrest and fragmentation. Movements of the first category, associated with a high quality sleep, are reduced in aged people, while movements of the second category, associated with a lower quality of sleep, are increased.

CONCLUSIONS

The number of body shifts and turnings in nocturnal sleep tend to decrease in the, more fragmented, sleep of elderly people. On the other hand, small movements are increased, presumably due to the decrease in quality of sleep, expressed in lower sleep efficiency and in an increased sleep fragmentation.

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THE INFLUENCE OF REAL - WORLD STRESS ON ADOLESCENTS' SLEEP

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INTRODUCTION

Insufficient and poor sleep are common problems during adolescence. In the adolescent population the prevalence of sleep quality or insomnia range from 11% to 47% [1,2]. Concerning sleep duration or Time in Bed empirical evidence demonstrates that approximately 45% of adolescents sleeps less than the required nine hours per night [3].

Previous research demonstrates that sleep and the endocrine stress system influence each other. From animal studies comes evidence that reduced sleep over a longer time period (chronic sleep reduction) increases the activity of the major neuroendocrine stress systems, such as the autonomic sympatho-adrenal system and the hypothalamic-pituitary-adrenal (HPA) axis [4]. Chronic sleep reduction not only affects the baseline activity of the stress system, but it also alters its response to a subsequent stressor [5]. Chronic sleep reduction in animals leads to gradual changes in brain systems that are involved in the regulation of stress responses [4,5].

Findings from human studies are in line with the above described animal studies as they show that higher cortisol levels, a HPA axis marker, are related to shorter subjective and objective sleep durations and poorer sleep quality [6]. Individuals' perceived stress is found to be associated with an increased risk of experiencing poor sleep[7]. Moreover, results, coming from a study that examined the influence of real-world stress on the restorative function of sleep in college students, revealed that anxiety about examination, which can be seen as a stressful situation, is accompanied by suppression of the cardiorespiratory resting function during sleep [8].

Adolescents are often confronted with high demands concerning cognitive and school performance causing daily life stress especially before and during exam weeks. However, to date no study has been conducted investigating adolescents' objectively measured sleep patterns in stressful situations and situations without stress. This study examines the influence of real-world situations that are characterized by low (week without exams) and high stress (week prior to the exams/ exam week) on adolescents' sleep.

METHOD

Procedure

Participants were recruited in June and September 2009 from two different schools in Amsterdam, the Netherlands. Active informed consent from schools, parents, and participants were obtained. Adolescents filled in questionnaires about demographic data and general stress level in a non-demanding situation at school. Each individual received an actiwatch and a personal account for an online sleep diary. The sleep was monitored three

weeks long for school nights using AW4 actiwatches. Participants registered each day their bedtimes and getup times in the online sleep diary. The third week was the exam week, which is assumed to be characterized by more stress than the first (baseline) week.

Measurements

Sleep. Actigraphy involves use of a wristwatch-like portable device that can record movements over an extended period of time (e.g. a few weeks). Actigraphy is known to be a reliable and valid measure to study sleep parameters in a natural environment [9]. Participants were instructed to wear the actigraph on their nondominant wrist when preparing for sleep and remove it in the morning after they got up. Two different dimensions of sleep were examined: (a) sleep duration: Difference between sleep end and sleep onset, and (b) sleep efficiency: Percent of uninterrupted night sleep, reflecting an objective aspect of sleep quality. In order to get as reliable data as possible, we visually examined the actigraph data. If the data received from the sleep diaries did not match the visual inspection we applied the following general rule: If the bedtime from the sleep diary was set at a time point at which it was obvious that the individual was already sleeping we set it back to the first peak before the dropoff. If the bedtime from the sleep diary is earlier than expected from the actiwatch data we would only correct in cases in which it is clear that the actiwatch is not worn at this time point. Otherwise we would not correct the actiwatch data as somebody could also be lying in bed without moving. If the getting up time was set at a time point at which it was obvious that the individual was still sleeping we corrected the data by changing the getting up time to the following peak. If the getting up time from the sleep diary was later than the objective data we only corrected the data in cases in which it was obvious that the actiwatch was not worn any more.

Stress Level. General stress level was measured with the Stress Questionnaire for Children [10]. This questionnaire consists of 17 items (e.g. I am often in a hurry; I am tensed; I get easily upset) being rated on 4-point Likert scales (1 = *this is not at all true for me*; 4 = *this is true for me*). Cronbach's alpha was .77.

Statistical analysis

All data were analyzed with the statistical package SPSS 15.0. Linear Mixed Models were used in order to investigate differences in sleep duration and sleep efficiency across time. Main and interaction effects for gender and general stress level were investigated by adding these variables to the model.

RESULTS

Participants

76 adolescents (31.4 % boys; mean age: 15.28 years) from two different schools in Amsterdam participated in the study. Participants from the two schools did not significantly differ with regard to age, gender and sleep variables (all $p > .05$).

Sleep and stress

Preliminary results reveal that in comparison to the first (baseline) week adolescents slept worse during week two (B = -.72, SE = .37, $p < .05$) and three (B = -.80, SE = .38, $p < .05$), indicating that stressful times negatively influenced their sleep efficiency. Sleep efficiency was reduced from 81.04 % at baseline to 79.56 % during the second and to 79.60 % during the third week. Sleep duration was extended from 8:08 hours/night during the first to 8:23 hours/night during the second (B = .15, SE = .08, $p < .05$) and 8:26 hours/night during the

third week (B = .19, SE = .08, $p < .05$). Means and standard deviations for the sleep characteristics for all three weeks are given in **Table 1.** The effects during the second week were larger for students with high baseline stress levels (B = .03, SE = .01, $p < .05$). No gender effects were found.

Table 1. Means (standard deviation) of sleep characteristics across time

	week 1	week 2	week 3
sleep efficiency (%)	81.04 (5.98)	79.56 (6.76)	79.60 (6.14)
sleep duration (hours/night)	08:08 (1.92)	08:23 (1.21)	08:26 (1.15)

Note. N = 76

DISCUSSION AND CONCLUSION

This study shows that stressful times had a negative influence on adolescents' sleep efficiency. The reduction in sleep efficiency combined with the extended sleep duration during the stressful weeks suggest that individuals experienced more intermittent wakefulness, meaning that their sleep was more fragmented during the more stressful weeks. Longer sleep durations during week two and three could result from a different school start time schedule during this time period being supported by the finding that differences were found in sleep end rather than in sleep onset. Future research should be done in a larger sample in order to support the findings of this study. Furthermore, results from sleep diaries could give more important insight into the effects of stress on participants' subjective sleep experience.

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SIMULTANEOUS ASSESSMENT OF CHANGES IN SLEEP, NEUROPHYSIOLOGY AND NEUROCHEMISTRY FOLLOWING PSYCHOSOCIAL STRESS IN RATS

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INTRODUCTION

Depression is a heterogeneous disorder often manifested by symptoms at psychological, behavioural, neurophysiological and neurochemical levels. Affective disorders are known to be potentially developed and aggravated when humans are subjected to uncontrollable, psychological factors related to social stress of varying severity and nature (e.g., lack of social support). Animal models using various (more physical) stressors, such as footshock, restrain or forced swim have been developed to study the mechanisms underlying stress-induced neurobiological disturbances. However, these stressors bear little resemblance with natural stress conditions and the validity of these animal models of depression has been questioned. Manipulations of social factors in socially living animals may strongly impact the physiology and behaviour in rats or mice. Consequently, several animal models have been developed that use social stress to investigate questions related to the aetiology, treatment and prevention of depression in mice[1], rats[2,3], tree shrews[4] and non-human primates[5]. On one hand, some of the rodent models use the social status of an individual rat within an established group of rats housed together as an index of social defeat[6], however a major concern with this social status model is the variability inherent to complex social interactions[7]. On the other hand, a resident-intruder protocol based on the interaction of 2 rats i.e. a dominant male defeating an unfamiliar male intruder seems better to control[3]. The resident-intruder paradigm have repeatedly shown to produce behavioral, physiological and endocrinal alterations in the subordinate animal alike those observed in depression: including a loss of hedonic responsiveness[8], changes in the daily rhythm of temperature, heart rate and activity[9], decreased social interaction and weight loss, as well as alterations in the reactivity of the hypothalamic-pituitary-adrenal axis, the major endocrine component of the stress response[10].
In our experimental approach, we first established that the social defeat procedure induced a classical stress response on various behavioral and physiological parameters. Next, we focused on concomitant changes in sleep-wake architecture, electroencephalographic dynamics, behavioural, endocrinal and extracellular levels of monoamines in prefrontal cortex and hippocampus nuclei in subordinate defeated 'stressed' rats during the conditions of repeated social stress caused by a dominant conspecific.

METHODS

Animal and Surgery

Adult male Sprague-Dawley (SD) rats obtained from Charles River (Sulzfeld, Germany) and weighing 347 $\pm$ 23 g at the beginning of the experiment, were used as intruder rats. Animals were individually housed in Macrolon cages type II, which were placed in individually,

ventilated cage racks. Male Brown Norway rats, supplied by Janvier (Le Genest-St-Ilse, France), weighing at least 400 grams at the time of the experiment, were used as dominant, resident rats. Each dominant male was housed together with a female in IVC racks holding Macrolon type III cages. Housing and experiments were done in a controlled environment with room temperature at 22 ± 2°C and relative humidity at 60%, a reversed 12:12 light-dark cycle (lights on from 06:00 p.m. to 06:00 a.m.; light intensity: ~ 100 lux) and food and water available ad libitum. All animal procedures were approved by the institutional animal care and use committee.

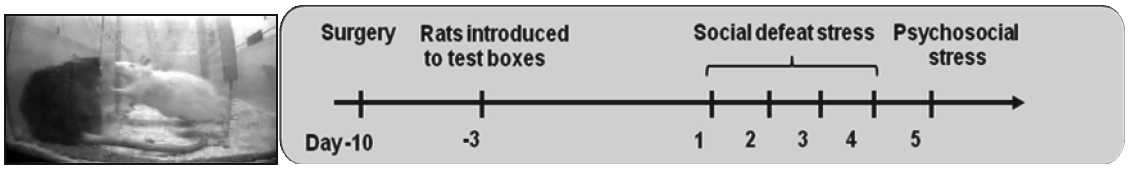
Under isoflurane inhalation anaesthesia, intruder rats were instrumented with a biopotential telemetry probe (Data Sciences International) with a catheter inserted in the descending aorta for recording cardiovascular parameters and EEG leads placed at AP + 0.2, L –0.2 and AP –0.6, L –0.3, from bregma for recording the fronto-hippocampal EEG. Neurochemical variables were measured via a guide cannulae implanted unilaterally into the PFC (+ 3.0 mm rostral, - 0.75 mm lateral, - 0.75 ventral from bregma) and HIP (-5.25 mm caudal, - 5.0 mm lateral, - 3.5 mm ventral to bregma)

Apparatus

The experiment was performed in a sound-attenuated box equipped with two digital cameras for continuous behavioral recordings, a microinjection pump and liquid swivel mounted to a counterbalanced arm for microdialysis sampling as well as a receiver for telemetric recordings. White and dim red lights were controlled according to the light-dark cycle in the holding room (red light on from 06:00 a.m. to 06:00 p.m. and white lights from 06:00 p.m. to 06:00 a.m.).

Experimental design

The repeated social stress condition consists in social stress interaction for 1 hr during five times a week (study design below). The control animals were placed in the novel clean cage, while the intruder animals were placed in the cage of a resident aggressive conspecific Brown Norway rats. During the 4 consecutive days of the experimental procedure, the defeated animal had an olfactory and sensory contact through a wire mesh insert grid that was removed for 5 minutes to allow confrontation (social defeat stress) at random time within the 1 h interaction. On day 5 the intruder rat remains 1 h in the insert cage to allow visual, olfactory and acoustic, but no physical contact (psychosocial stress). After each stress session, animal were weighed to evaluate the impact of successive stress exposure. As a control procedure, Sprague Dawley rats were placed in novel empty cages and followed similar experimental conditions to those of the defeated rats.



On day 5 (psychosocial stress), microdialysis probes were introduced into the prefrontal cortex (PFC) and hippocampus (HIP) of rats to collect samples each 30 min. The average of the first three samples was taken as baseline samples. The next three samples were referred to stress phase, and three additional samples were collected under baseline conditions (post stress phase). From the HIP samples, 15 µl were considered for electrochemical analysis of serotonin (5-HT); the remaining 35 µl were later subjected to radioimmunoassay for corticosterone analysis. 15 µl from the PFC were used for electrochemical analysis of dopamine (DA) and norepinephrine (NE).

Sleep and EEG variables

For sleep-wake cycle recordings the continuous 24-h EEG signals over 6 days of the experimental procedure were scored per 10-second epochs averaged to 30 minute periods as being active wakefulness (AW), quiet wakefulness (QW), light slow wave sleep (lSWS), deep slow wave sleep (dSWS), or rapid eye movement (REM) sleep.

Absolute EEG power spectra dynamics that occur when an intruder animal is confronted with a dominant resident rat were calculated as a total score for five different frequency bands: 0.4 to 4.0 Hz (δ), 4.2 to 8.0 Hz (θ), 8.2 to 12.0 Hz (α), 12.2 to 14.0 (σ), 14.2 to 30.0 (β) and 30.2 to 60 Hz (γ).

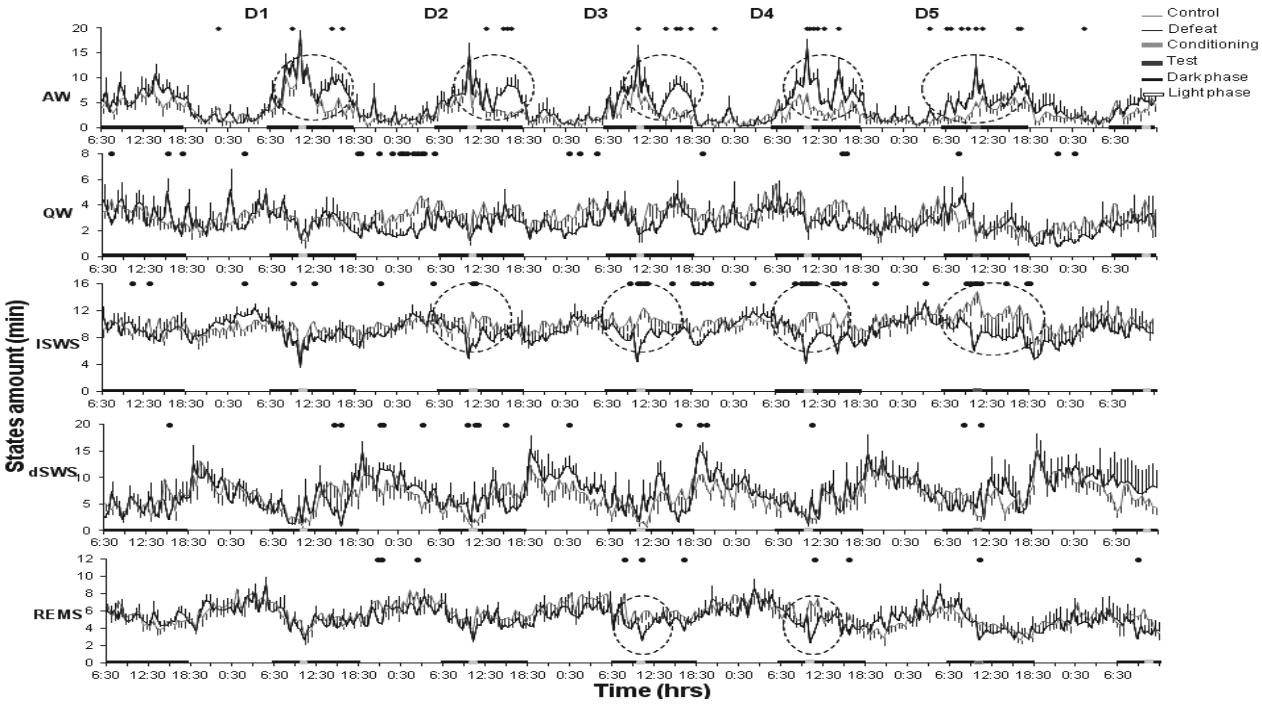
Statistical analysis

Values of different vigilance states were averaged into 30 min periods over the dark and light period. The EEG power spectra are expressed as mean absolute power in different frequency ranges and presented as mean values for each group. A Wilcoxon-Mann Whitney rank sum test was applied and value of $p<0.05$ is considered to be significant. DA and 5-HT levels are expressed % changes of baseline in prefrontal cortex and hippocampus, during or after psychological or novelty stress. All data were expressed as mean values ± S.E.M.. A Friedman repeated measures followed by Dunn's test were applied to assess significant differences between groups.

RESULTS

Sleep-wake architecture

Defeated rats spent significantly more time in quiet wakefulness at the expense of reduction in time spent in active wakefulness during the interaction period as well as during the second half of the dark period. Concomitantly, a significant enhancement in time spent in light sleep during and immediately following the stress procedure was found in stressed animals. Additionally, a tendency for an increase in REM sleep was observed with a difference found in the number of periods being significantly higher for defeated rats during the dark and light phase (Figure 1).



1: Effects of repeated exposure to social defeat stress (days D1 to D4) and psychosocial stress (D5) on sleep-wake architecture in rats. Data are mean values ± S.E.M. expressed in minutes for the states of active wake (AW), wake (QW), light slow wave sleep (lSWS), deep slow wave sleep (dSWS), and rapid eye movement sleep (REMS). • $p<0.05$ significance between control (n=8) and defeated animals (n=7).

Effects of social stress on EEG dynamics

In the control group, an overall increase in the EEG power was found when animals were introduced into novel cages with a marked increase in body temperature and a decrease in locomotor activity. Whereas in subordinate animals, alterations in brain activity were associated with a decrease in the EEG power over the frequency range 0.5-60 Hz and a sharp transient increase in slow delta wave activity.

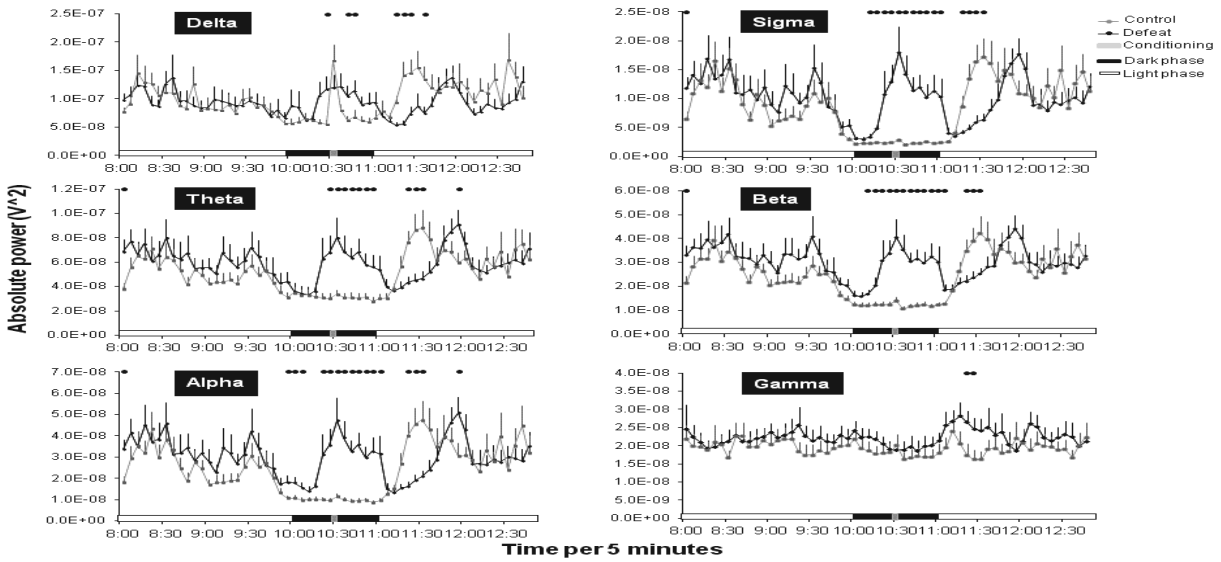


Figure 2: Changes in the absolute EEG power activity during exposure to social defeat stress in rats. • $p<0.05$ significance between control (n=8) and defeated animals (n=7).

Effects of psychosocial stress on monoamine levels

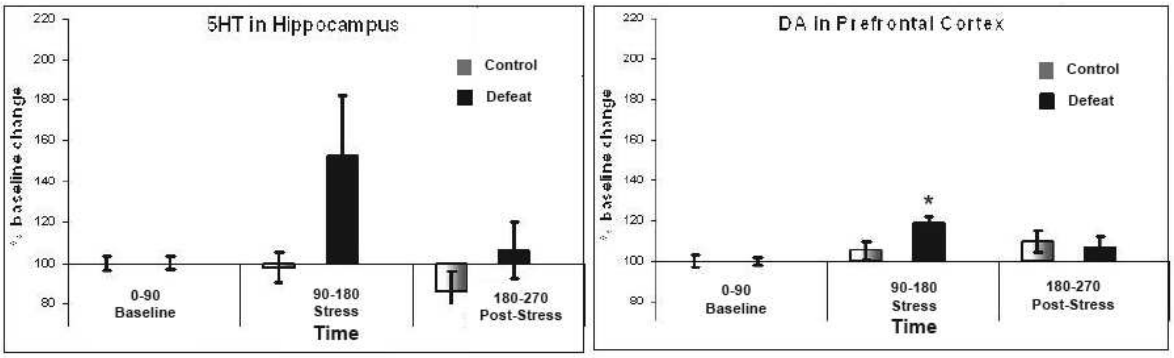


Figure 3: 5-HT and DA release before (baseline conditions), during and after psychosocial stress (n=7) or novelty for controls (n=8). Data are expressed as % changes of baseline, in hippocampus and prefrontal cortex, respectively and presented as mean values ± S.E.M.

Psychosocial stress consistently enhanced the release of 5-HT in the hippocampus (left panel) and DA in PFC (right panel) (Figure 3).

DISCUSSION

The present study showed that repeated exposure to social stress procedure induced consistent changes in a wide range of physiological and behavioural parameters, including activation of hemodynamic parameters, decreases in general body activity, hyperthermia, increased corticosterone level, freezing and submissive behaviour (data not shown). Most of these responses were short lasting and returned to basal levels after termination of social interaction. In addition, defeated animals exhibited an important anticipatory rise in body temperature that is dissociated from locomotor activity.

Our findings extend earlier observations in rodents exposed to social defeat stress, and demonstrate that repeated confrontation with an aggressive dominant conspecific produced marked loss of body weight[11], alterations in the daily rhythms of body temperature and locomotor activity[3], increase activation of the cardiovascular system and neuroendocrine response[10].

The increased need for sleep in duration as well as in efficiency is related to the nature of waking experience. Neuroendocrine changes after stress experience results in disturbance of sleep process, which are often associated with immediate reduction in the duration of NREM sleep and/or REM sleep followed by subsequent sleep rebound. However, the response of animal subjected to social defeat stress varies with the magnitude and quality of stress experience. The sleep debt may particularly be associated with an increase of the slow wave activity during NREM sleep without consistent changes in the duration of such vigilance state or sleep time[12]. However, social stress may also affect the duration of specific vigilance states as shown in great reduction in the duration of REM sleep in mice[13]. Our findings indicate that social stress reduces active waking, enhances light sleep and induces a trend for increased of REM sleep. These data provide evidence of an ongoing development of disturbance in sleep-wake architecture.

An overall increase in the EEG power was found when animals were introduced into a novel cage. Whereas, in subordinate animals, alterations in brain activity were associated with a marked decrease in the EEG power over the frequency range 0.5-30 Hz and a sharp, transient

increase in slow wave activity. This major novel findings in defeated subordinate animals i.e. a remarkable flattening of the EEG power between 0-30 Hz was accompanied with a marked increase in body temperature and a decrease in locomotor activity; a dissociation that in view of the EEG changes may reflect increased brain activity and metabolism. However, such EEG alterations disappeared at the end of the stress conditions. Furthermore, psychosocial stress leads to an increase in serotonin and dopamine levels. This transient stimulation of the monoamine systems such as 5-HT and or dopamine have been hypothesized to impair the permeability of the blood brain barrier and consequently can result in the flattening of EEG activity as it was described in long-term immobilisation stressed rats[14].
Both correlational and differential changes as quantified simultaneously within subjects in this study emphasize the importance of assessing the processes underlying sleep and stress dynamics in a parallel systems neuroscience approach. These changes can further be evaluated as potentially useful (bio)markers in the aetiology and/or treatment of stress-related aspect of psychiatric disorders such as depression.

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TIME ON TASK EFFECT IN REACTION TIMES DURING A SIMULATED DRIVING TASK

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INTRODUCTION

The time-on-task effect entails a decrement in cognitive performance across the duration of a performance task.[1,2] The Psychomotor Vigilance Test (PVT)[3] is sensitive to the time-on-task effect, showing a lengthening of average response time (RT) and an increase in RT variability in as little as 10 min, especially under conditions of sleep deprivation.[2,4,5] The PVT is a simple reaction time task in which subjects press a button when a stimulus appears on a computer screen.[3] It is believed that high stimulus density and the associated continuous requirement for sustained attention are what make the PVT particularly sensitive to the time-on-task effect.[2,4]

The time-on-task effect has also been observed during simulated driving tasks. In one driving simulator study,[6] steering performance appeared to deteriorate during a 30 min driving task. In another driving simulator study,[7] steering wheel movements increased across the duration of a 40 min driving task performed twice (once under monotonous conditions and once under more varied conditions), which suggested a time-on-task effect in steering performance.

Drews et al.[8] used a driving task on a high-fidelity driving simulator to investigate the effect of distracted driving on driving performance. The task, which was approximately 30 min in duration, involved following a leading car, and braking when, from time to time, the leading car decelerated (as indicated by its brake lights). An overall slowing of braking RTs during distracted driving was observed relative to a non-distracted control condition.[8]

Both the simulated driving task of Drews et al.[8] and the PVT appear to require sustained attention to produce motor responses to visual stimuli across the duration of the task. The research reported here sought to adapt the simulated driving task to make it more closely resemble the PVT, so as to achieve high sensitivity to the time-on-task effect (in braking RTs) while maintaining the face validity associated with high-fidelity driving simulation.

METHODS

High-fidelity driving simulator. A high-fidelity PatrolSim IV driving simulator (MPRI, Salt Lake City), which is widely used to train professional drivers, was employed. The simulator has three screens that form a 180º angle of view. These serve to project the road through simulated windshield and side windows around the driver. A rear-view mirror and two side-view mirrors are also projected on the screens, and the scenery in the windows and mirrors is updated in real time. The simulator has an open-seat driver cockpit with an automatic transmission design. It includes a car seat, steering wheel, brake and gas pedals, lever to select the operating mode, and full dashboard. The simulator imitates the dynamics of a real car, including interaction with the road. The steering wheel provides feedback from the simulated wheels by subtle movements; however, the rest of the cockpit does not move. We

installed hardware and software for capturing driving performance data (sampled at 60 Hz), in order to adapt the simulator for research use.

Simulated driving task. In the driving task, subjects drove a simulated sports sedan on a 2 to 3 lane freeway, set in a sunset environment for optimal visual contrast of the leading car's brake lights. Subjects were asked to follow a leading car, which was programmed to cruise at 64 miles/h, for 30 min. At set times during the drive, the leading car decelerated, turning on its brake lights. There were 90 deceleration events, spread out over the drive at inter-stimulus intervals of 10–30 s (uniformly randomly distributed in 4 s increments). Subjects were required to press the brake pedal of the driving simulator as soon as they observed the brake lights of the leading car. The leading car then accelerated back to its cruising speed,[1] and the subjects were to resume following the leading car. Subjects were instructed to maintain a safe driving distance behind the leading car. If they could read the leading car's license plate, they were too close. They received a message on the center screen if they were more than 100 m behind the leading car, which was too far. There was no other (simulated) traffic.

RTs were calculated from the onset of the deceleration of the leading car until the moment the brake pedal was pressed by the subject. On a few occasions (four times in total), subjects pressed the brake pedal before the start of a deceleration event. For the present study, these were treated as missing data.

Other neurobehavioral measures. A 20 min PVT was administered prior to the driving task, and a 10 min PVT was administered following the driving task. These PVTs were performed while in the car seat of the simulator, using software implemented on a Palm Centro smartphone.[9] Visual stimuli were presented on the display at 2–10 s response-stimulus intervals (uniformly randomly distributed in 1 s increments). One outlier RT (greater than 10 times the mean), occurring during the first 10 min of the pre-driving PVT, was discarded.

Before the pre-driving PVT and after the post-driving PVT, subjects filled out the Karolinska Sleepiness Scale (KSS).[10]

Experimental procedures. Subjects first completed a screening session in the laboratory, during which they performed a 5 min simulated practice drive to introduce them to the simulator and the basic aspects of the simulated driving task. Between 1 and 23 days after their screening session, subjects attended a laboratory experimental session of about 2 h duration in the morning (at either 09:00 or 11:00). At the beginning of the session, they again performed the 5 min practice drive, filled out the KSS, and took a 15 min break. They then performed the 20 min PVT, carried out the 30 min simulated driving task, performed the 10 min PVT, and filled out the KSS once more. During the 24 h prior to the experimental session, subjects were not allowed to drink caffeine or alcohol.

Subjects. Eleven subjects (7 men, 4 women) aged between 21 and 41 y (mean ± s.d.: 28.9 ± 7.4 y) completed the study. One additional subject experienced symptoms of simulator adaptation syndrome during the driving task, and withdrew from the study. All subjects were in possession of a valid driver's license, had normal or corrected-to-normal vision. They were screened for absence of medical conditions, sleep disorders, psychological disorders, and current alcohol abuse, and they reported not having abused any drugs within the past year.

Statistical analyses. Simulated driving task braking RTs and PVT RTs were grouped in consecutive 10 min blocks, and the raw RTs were then analyzed with mixed-effects analysis of variance (ANOVA) to test for an effect of block. Analyses of interest involved comparing

[1] Shortly after commencing acceleration, the leading car sometimes braked in order not to overshoot its cruising speed. This resulted in a few additional (unintended) deceleration events, which made the inter-stimulus intervals to the next (intended) deceleration events a few seconds shorter than the programmed 10–30 s range values. We deliberately selected the programmed range such that, based on the literature,[12] the shortened foreperiods would not become so brief that they would confound RTs with refractory period effects.

the first through third 10 min blocks of the simulated driving task; comparing the first and second 10 min blocks of the 20 min pre-driving PVT; and comparing the first 10 min block of the 20 min pre-driving PVT to the (only) 10 min block of the post-driving PVT. Furthermore, pre- and post-testing KSS scores were compared, using a paired *t* test. We hypothesized that RTs on the simulated driving task, RTs on the PVT, and sleepiness scores on the KSS would all increase over time in the experimental session.

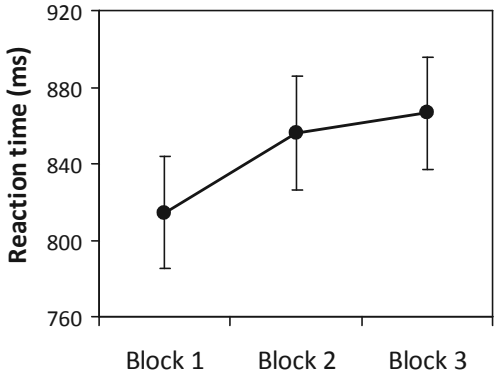

Figure 1. Braking RTs (mean ± s.e.) over the three 10 min blocks of the simulated driving task, reflecting a significant time-on-task effect.

RESULTS AND DISCUSSION

There was a significant effect of block on the simulated driving task ($F_{2,973} = 6.5$, $P = 0.002$); average RTs became progressively slower towards the end of the 30 min task (Figure 1). There was also a significant effect of block on the 20 min pre-driving PVT ($F_{1,2067} = 23.9$, $P < 0.001$); average RTs were significantly slower (by 19.7 ms) during the second 10 min block than during the first 10 min block. RTs during the 10 min post-driving PVT were slower yet (by another 9.2 ms), and significantly different from the first 10 min of the 20 min pre-driving PVT ($F_{1,2073} = 11.4$, $P < 0.001$). Post-driving KSS scores were significantly higher (by 2.1 units on a scale from 1 to 9) than pre-driving KSS scores ($t_{10} = –4.6$, $P = 0.001$).

These results are consistent with our hypotheses. There was a significant time-on-task effect in the simulated driving task (Figure 1). As expected, there was also a significant time-on-task effect in the pre-driving PVT. This effect appeared to persist for 30 min (i.e., during the driving task) and to carry over to the post-driving PVT. Thus, unlike what has been reported in other task-switching paradigms,[11] in this study interspersion of the PVT with another task did not provide relief of the PVT time-on-task effect, but rather seemed to maintain (or perhaps even enhance) it. The build-up of cognitive fatigue over time was experienced subjectively as well, as evidenced by the increase in KSS sleepiness scores.

A recent theory posits that the time-on-task effect may stem from activity-dependent induction of a sleep-like state in cortical columns used during the performance task at hand.[2] In this view, the observation that post-driving PVT performance was degraded relative to pre-driving PVT performance implies that the intervening 30 min driving task entailed continued activation of cortical columns used during both the PVT and the driving task, perhaps related to a common attentional system in the brain. This supports the notion that the PVT captures cognitive deficits—implicated in sustained attention[4]—that are also relevant for automobile driving (and vice versa).

CONCLUSIONS

Building on the work of Drews and colleagues,[8] we successfully developed a simulated driving task that shows sensitivity to the time-on-task effect. Implemented on a high-fidelity driving simulator, the new task has a high level of face validity. It involves frequent braking while driving at relatively high speed, and demands sustained attention. This also occurs in real-world driving, such as during bad weather, on roads with potholes, and in busy traffic.
The time-on-task effect is similar to the effect of sleep deprivation on cognitive performance,[4] and the two effects exacerbate each other.[2,4,5] We therefore plan to further validate the task in a sleep deprivation study. Our ultimate goal is to establish the task as a cognitive assay that will be widely recognized as directly relevant for real-world operational impairment.

ACKNOWLEDGMENTS

We thank Frank Drews for sharing with us the simulated driving task[8] that we adapted, and Roger Ratcliff for pointing out the task's similarity to the PVT. We also thank Jason Moore, Amy Bender, Luke Huang, Christopher Mott and Daniel Mollicone for help with the development of driving simulator procedures. This research was funded by CDMRP award W81XWH-05-1-0099 and DURIP grant N00014-08-1-0802.

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CAN SLOW MELATONIN METABOLISM BE ASSOCIATED WITH A SINGLE NUCLEOTIDE POLYMORPHISM IN THE CYP1A2 GENE? -A PILOT STUDY-

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INTRODUCTION

The majority of patients suffering from a biological clock sleep disorder have a Delayed Sleep Phase Syndrome (DSPS). In DSPS, patients tend to fall asleep later than desired and / or have difficulty waking up in the morning. Usually, in such cases, the Dim Light Melatonin Onset (DLMO) has shifted to a later moment. Exogenous melatonin is able to advance the endogenous melatonin rhythm and its associated circadian rhythms, including sleep-wake, temperature and cortisol rhythms, maximally when it is administered 5 hours before DLMO[1]. Melatonin in the usual pharmcotherapeutic doses (1-5 mg), cannot be found in blood or saliva within 12 hours after its administration[2]. When exogenous melatonin is metabolized slowly it will remain present 24 hours after its administration. Subsequent doses of melatonin will give a steady-state melatonin level. Consequently, exogenous melatonin loses its chronobiotic effect as the 24 hour melatonin rhythm disappears resulting in the return of the sleep disturbance after several weeks of treatment. A metabolisation test performed in suspected slow metabolizing patients showed that high levels of melatonin were the probable cause of the disappearing effectiveness of exogenous melatonin. This might be caused by decreased activity / inducibility of the CYP1A2 enzyme[3]. Melatonin is metabolized in the liver by Cytochrome P450 1A2 (CYP1A2) to its primary metabolite 6-hydroxymelatonin, conjugated with a sulphate group and subsequently excreted in urine. About 90% of melatonin is metabolized by CYP1A2 and in a lesser extent by CYP2C19, CYP1A1 and CYP1B1. Several reports indicate that a Single Nucleotide Polymorphism (SNP) in the CYP1A2 gene might cause decreased activity or inducibility of the CYP1A2 enzyme[4]. Putative allelic variants with decreased activity or inducibility are: CYP1A2*1C, CYP1A2*1K, CYP1A2*3, CYP1A2*4 and CYP1A2*6[5]. The CYP1A2*1F allelic variant is associated with a higher inducibility[6].
In this clinical pilot study we investigated whether a specific SNP in the CYP1A2 gene can be associated with slow melatonin metabolisation.

METHODS

Patients participating in this study underwent a metabolisation test: patients started at 12 PM (t=0) with collection of the first salivary sample immediately followed by oral intake of melatonin (1 mg Fagron BV, The Netherlands). Additional salivary samples were collected at 2 PM (t=2), 4 PM (t=4) and 8 AM the following morning (t=20). Patients were asked to refrain from coffee and intensive exercise during the test. Saliva was collected with

Salivette® (Sarstedt, Germany). Inclusion criteria were: patient requiring melatonin for treatment of DSPS. Patients were between age 6 and 60. Exclusions criteria were: known liver disease and known hypersensitivity for melatonin.
Melatonin levels were measured using a Radioimmunoassay (RIA) (Buhlmann laboratories, Switzerland), as previously described[7]. Melatonin measurements were performed and DNA was extracted as previously described[8]. PCR experiments were performed on the real-time PCR cycler Rotor-Gene Q (Qiagen Benelux BV, The Netherlands). Sample handling was performed with the QIAgility System (Qiagen Benelux BV, The Netherlands). PCR chemistry was in accordance with manufacturer's specification except for the increased template volume of 5.25 µL. Template and 2.75 µL Master-Mix solution was pipetted into a gene-disc 72 giving a total PCR volume of 8 µL. The 2.75 µL Master-mix solution consisted of 2.5 µL Taqman® Genotyping Master-mix and 0.25 µL Taqman® Drug Metabolism Genotyping Assays (both supplied by Applied Biosystems BV, The Netherlands). The assays consisted of unlabeled PCR primers and Taqman® MGB probes (FAM™ and VIC® dye-labeled for the detection of allele 1 and allele 2) with assay number: C_15859191_30, C_30634146_10, C_34816147_10, C_30634246, C_30634244_20 and C_8881221_40. These assays detect a substitution in the CYP1A2 gene at position -3860 G>A, -729 C>T, 2385 G>A, 2499 A>T, 5090 C>T and -163 C>A, respectively. Detecting the following CYP1A2 variants respectively; *1C, *1K, *3, *4, *6 and *1F. The thermal cycler conditions were: initial step of 10 minutes at 95°C followed by 50 cycles of 15 seconds at 92°C (denature) and 90 seconds at 60°C (anneal/extend).
SPSS v17 (SPSS Inc. Chicago, Illinois) was used for descriptive statistics. P-values were determined using Fisher-Exact.
This study has been approved by the Gelderse Vallei Hospital review committee for scientific research (BCWO number: BC/0903-111). Informed consent was obtained from the patient or its parent / guardian.

RESULTS AND DISCUSSION

Out of a 100 patients we obtained material for DNA extraction and melatonin measurements from 78 patients. In 10 of these patients the melatonin level in the first sample was above 15 pg/mL and therefore omitted from further calculations. The sample amount of 5 patients was too low for melatonin measurements and 1 patient was outside the age range. Further calculations were done with the remaining 62 patients. In 69.4% cases melatonin levels were above the linear range of 0.5 – 50 pg/mL after administration (at t=2) of exogenous melatonin. At t=4, 25.8% of the samples were above the linear range and at t=20, 6.5% were above the linear range. One patient had a heterozygote CYP1A2*1C. CYP1A2 variant; *1K, *3, *4, *6 were not detected. CYP1A2*1F genotype results are presented in Table 1 and Figure 1. These yield an indication of the pharmacokinetic profile of melatonin within the CYP1A2*1F C/C, A/C and A/A genotypes.

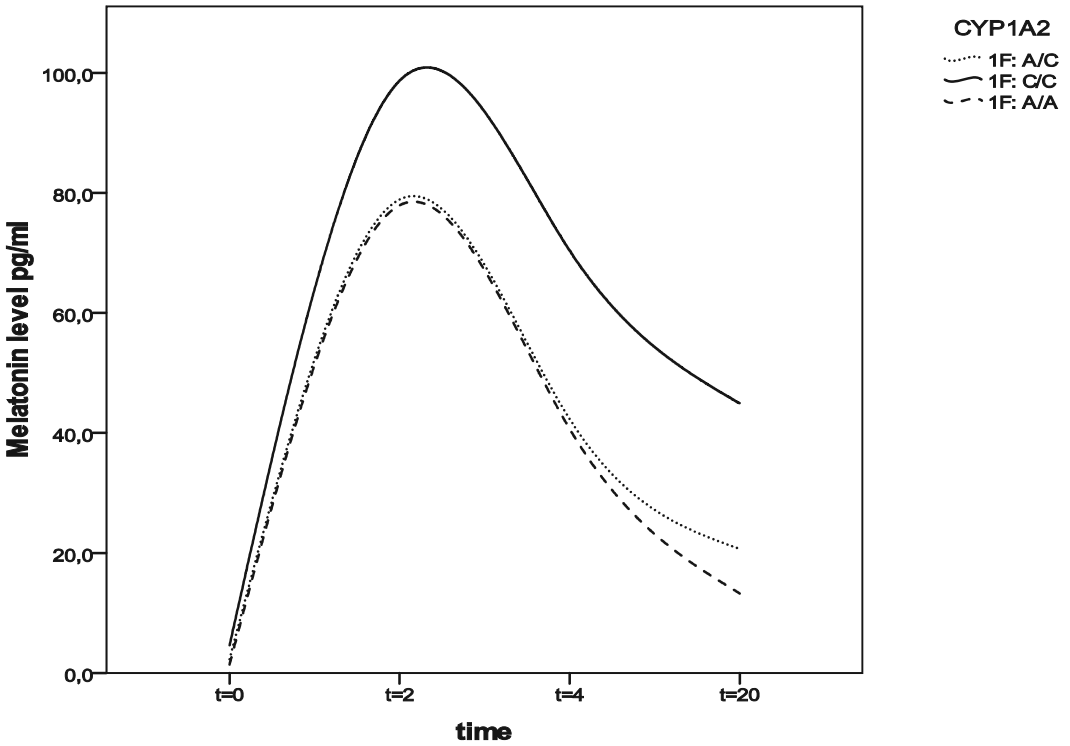

Figure 1: The pharmacokinetic profile of CYP1A2*1F C/C, A/C and A/A genotype after administration of 1 mg of melatonin.

A threshold melatonin value of 37.5 pg/mL was used to group the different CYP1A2*1F genotype. The threshold was set as follow: the mean melatonin value of CYP1A2*1F A/A sample size + 3 SD (at t=20: mean 10.61 pg/mL, SD 8.96 pg/mL). The percentage of patients above the threshold level was calculated (see Table 1)

Table 1. Comparison between CYP1A2*1F allele and melatonin levels at t = 20

*CYP1A2*1F genotype*	*Number of patients with melatonin ≥37.5 pg/mL*	*Number of patients with melatonin <37.5 pg/mL*	*Percentage*	*P-value*	
C/C	2	3	40.0 %	0.04	n = 5
A/C	3	22	12.0 %	0.19	n = 25
A/A	1	31	3.1%	-	n = 32
Total	5	57			62

P-value calculated using Fisher-Exact compared to A/A genotype

This study shows that among carriers of the CYP1A2*1F C/C genotype a significantly higher percentage of patients have melatonin levels above the threshold level (≥37.5 pg/mL) compared to the CYP1A2*1F A/A.

CONCLUSIONS

This pilot study indicates that the CYP1A2*1F C/C genotype could potentially be associated with slow melatonin metabolism. The results in this study warrants the start of a follow up in a larger study population.

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EFFECTS OF SLEEP-WAKE STATES ON THE N150 OF THE AUDITORY EVOKED POTENTIAL FROM THE RAT AMYGDALA

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INTRODUCTION

The amygdala is a collection of nucleï in the medial temporal lobe involved in the detection and processing of (potential) threatening events and the generation of behavioral, physiological and endocrine reactions in response to threats, commonly referred to as 'fear'.[1] Abnormalities in amygdala functioning have been reported in a number of anxiety disorders, such as phobias and post-traumatic stress disorder.[2] Understanding the neural basis of fear is thus potentially useful for the development of therapeutic interventions. Pavlovian fear conditioning has emerged as a widely used animal model for the study of the neural correlates of fear.[1] Typically performed in rats or mice, fear conditioning consists of pairing an intrinsic neutral stimulus such as a tone (conditioned stimulus, CS) with an aversive stimulus, typically a footshock (unconditioned stimulus, US). Conditioned fear responses towards the CS, such as freezing and increases in heart rate, develop rapidly, often within a few trials.

The amygdala is one of the main sites for neural plasticity underlying Pavlovian fear conditioning.[1] Lesions and temporary inactivation of the amygdala in rats block fear conditioning and human neuro-imaging reveals amygdala activation during fear conditioning.[3,4] Intra-amygdalar recordings of both single-units and field potentials show increases in the neural responses towards the CS.[3] These latter neural correlates are found mainly in the lateral nucleus of the amygdala.

In a number of studies we recorded CS-evoked Auditory Evoked Potentials (AEPs) from the lateral amygdala in freely moving rats during Pavlovian fear conditioning. We found that, compared to pre-conditioning baselines, a large negative AEP wave emerges during fear conditioning.[5-8] This slow component (start ~80 ms, end ~300 ms) reaches its peak amplitude at ~150 ms following stimulus onset and was therefore labeled N150. Increases in N150 amplitude are restricted to stimuli that evoke an increase in autonomic arousal such as a CS during fear conditioning and do not take place in response to stimuli that have behavioral significance but that do not evoke arousal, such as a CS predicting food reward.[8]

Although our previous results demonstrate a clear link between the N150 and emotional arousal, it is not known whether the N150 is also influenced by more general fluctuations in wakefulness and vigilance and in particular by states of lowered arousal such as drowsiness and sleep. As it is well known that the amplitudes of AEP components are influenced by sleep-wake states,[9,10] we sought to determine whether such influences also exist for the amygdalar N150. In order to test this we used EEG recordings obtained during the habituation protocol of a previous study.[8] Rats occasionally slept during these recordings and we obtained separate AEPs during wakefulness, drowsiness and slow-wave sleep (SWS).

METHODS

Adult male Wistar rats (n = 10) were used for this study. Stereotaxic surgery was carried out under inhalation anesthesia with isoflurane. Electrodes (Plastics One, MS333/2a, Roanoke, VA) were aimed at the right lateral nucleus of the amygdala according to Paxinos and Watson's atlas coordinates AP -3.60 mm, ML 5.20 mm and DV -8.30 mm.[11] EEG was referenced to a cerebellar recording. The electrode was attached to skull will acrylic dental cement and screws were placed to provide support. Rats were allowed one week of recovery prior to experimental procedures. Approval for surgical and behavioral procedures was obtained from the animal ethics committee of the Radboud University Nijmegen.

Conditioning chambers (25 x 25 x 40 cm), each located in a sound-attenuating cupboard, had a floor made of stainless steel rods (3 mm wide) for delivery of footshocks during fear conditioning. Two speakers placed in the left-side wall could produce a white noise stimulus for AEP triggering. EEG was band-pass filtered (1-100 Hz), amplified and then recorded with WINDAQ/Pro (DATAQ Instruments, Akron, OH) at 512 Hz.

Prior to fear conditioning there was a habituation phase in which white noise (85 dB, 15-s duration, 10-ms rise/fall time) was presented 200 times at an inter-stimulus interval varying between 10 to 30 s. EEG recordings obtained during this session were used for AEP analysis of different sleep-wake states in the present report. In a later experimental phase the white noise was used as a CS announcing a footshock (0.5 mA, 0.5 s). Two conditioning sessions of each 40 CS-US trials at intervals of 1-2 min were applied. Heart rate responses to the CS were used to verify successful conditioning.[8] Experiments were performed in darkness and conditioning chambers were cleaned with 70% ethanol after each use. After the experiment rats were perfused and the brains were removed for verification of correct electrode positions using a standard cresyl violet staining. All rats had electrodes positioned in or at the immediate border of the lateral amygdalar nucleus.[8]

Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) was used for EEG inspection and AEP averaging. A 50-Hz notch filter was applied offline. In each rat all 200 stimulus presentations in the EEG were classified as occurring during one of three sleep-wake states or marked for rejection because of EEG artifacts. Periods of slow-wave sleep (SWS) were easily identified by large delta oscillations in the EEG and periods of wakefulness were recognized by low-amplitude fast-frequency EEG (beta activity). Last, a state of 'drowsiness' was distinguished, identified by EEG of clearly larger deflections than the low-amplitude wakefulness EEG, but not as large and slow as during SWS. Eight out of ten rats had a sufficient amount EEG during SWS to obtain reliable AEPs. There were also eight rats for which drowsiness AEPs could be made (six of these also had a SWS AEP). Wakefulness AEPs were obtained in all ten rats and in order to exclude novelty effects averaging was restricted to the last 40 stimulus presentations, as described before.[8] EEG recorded during the fear conditioning sessions was used as a separate 'fear state' and AEPs were acquired for all ten rats.

In general, AEPs were obtained by cutting the EEG into segments ranging from 100 ms before, till 500 ms after stimulus onset. Each segment was baseline corrected relative to the 100-ms pre-stimulus period and segments were subsequently averaged. Given the broad shape of the N150, the mean amplitude in the 100-200 ms poststimulus time window was used for statistical analysis. Because different (numbers of) rats were included in the AEPs of the various sleep-wake states, no omnibus repeated measures ANOVA could be performed, instead we used t-tests for dependent samples.

RESULTS AND DISCUSSION

Figure 1 shows the AEPs obtained during the different sleep-wake states and during fear conditioning. Compared to wakefulness, the N150 was significantly reduced (less negative) during both drowsiness ($t(7) = -2.99$, $p < .05$) and SWS ($t(7) = -5.58$, $p < .01$; Figure 1B). The N150 disappeared during drowsiness and had a positive instead of negative polarity during SWS. The difference between drowsiness and SWS was also significant ($t(5) = -4.97$, $p < .01$). There was a substantial increase in N150 amplitude during Pavlovian fear conditioning compared to pre-conditioning wakefulness ($t(9) = 3.35$, $p < .01$). Figure 1B summarizes all statistical comparisons.

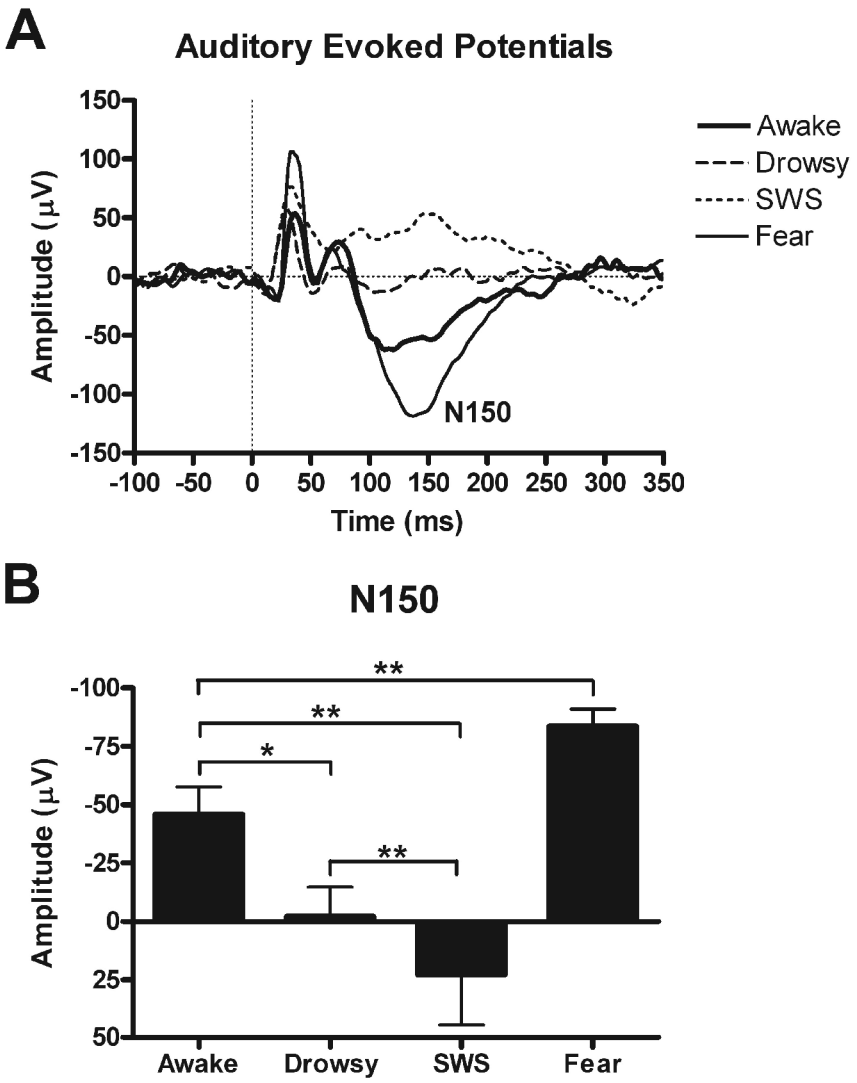

Figure 1. A: Auditory Evoked Potentials from the amygdala evoked by a white noise stimulus during states of wakefulness, drowsiness, slow wave sleep (SWS) and in a fear conditioning procedure in which the same stimulus served as a conditioned stimulus (CS) predicting foot shock delivery. B: Mean N150 amplitude in each of the four states of panel A. Error bars represent SEM. **, $p < .01$; *, $p < .05$.

These results demonstrate that an animal has to be in an awake state in order for a stimulus to elicit a N150. When an animal is no longer actively awake, as during drowsiness and SWS, the N150 disappears completely. Thus, wakefulness is a necessary condition for N150 evocation. Previous work by us repeatedly showed that the N150 increases during fear

conditioning compared to pre-conditioning recordings during wakefulness.[5-8] There is evidence that this increase is related to increases in emotional arousal, since CSs that evoke heart rate responses cause a N150 increase whereas as CSs that don't evoke such responses do not increase the N150.[8] Furthermore, the N150 decreases if a stimulus is repetitively presented alone in a habituation protocol and this decrement is correlated with decreases in basal heart rate.[7,8] Thus, our previous work suggests that the N150 is related to arousal and we now show that the N150 disappears during states that are the opposite of arousal, namely drowsiness and SWS. These results are in line with our hypothesis that the N150 is related to emotional arousal.

CONCLUSIONS

We determined the effects of different sleep-wake states on the N150 component of the AEP from the rat lateral amygdala, a component that is associated with emotional arousal responses. It was found that the N150 is present during wakefulness, but disappears during drowsiness and slow wave sleep (SWS). We conclude that a state of wakefulness is a necessary condition for the evocation of the N150 and that these results provide further support for the arousal hypothesis of the N150.

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THE ADDED VALUE OF DIM LIGHT MELATONIN ONSET IN DIAGNOSING IDIOPATHIC DELAYED SLEEP PHASE SYNDROME

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INTRODUCTION

Delayed Sleep Phase Disorder (DSPS) is a Circadian Rhythm Sleep Disorder. In the International Classification of Sleep Disorders (ICSD) DSPS is described as 'a persistent pattern of late sleep onset and late awakening times, with an inability to fall asleep and awaken at a desired earlier time'.[1,2] Patients suffer from sleep deprivation during the day, which results in educational and occupational problems. Opposing, on weekends or during holidays, when patients are not obliged to get up on a socially preferred time, they fall asleep at a 'late' bedtime, often past 2 a.m., and wake up 'late', without complaints of sleep maintenance or daily-functioning.[3] It appears the most common sleep-wake scheduling disorder and accounts for 10% of chronic insomnia patients.[4]
DSPS usually develops during adolescence, but sometimes in early childhood.[2] The pathophysiology of DSPS is still unclear, yet there are multiple hypotheses about its physiological or genetic basis. One of them suggests the influence of melatonin. Melatonin (5-methoxy N-acetyltryptamine) is secreted primarily by the pineal gland. Its concentration increased rapidly after the onset of darkness to a peak in the middle of the night and afterwards declines near one's habitual waking time. It is postulated that DSPS patients have a delayed onset of melatonin secretion through the night.[5] The onset of dim light melatonin secretion, or the dim light onset melatonin (DLMO) phase defines a 24 hour light/dark cycle in healthy individuals. It can be easily measured from saliva and therefore could be useful for assessing phase delays in patients suspected for DSPS.[5]
At this moment the diagnosis of DSPS is based on the clinical history of the patient. ICSD-criteria recommend polysomnography to rule out other diagnoses like snoring, sleep apnoea syndrome or periodic leg movement during sleep.[2] However, as many sleep disorders are presented with similar symptoms to DSPS, it is frequently misdiagnosed and treated inappropriately[6,7]. DSPS might be associated with several co-morbidities, i.e. ADHD[8,9] and chronic whiplash syndrome[10]. We restricted our study to idiopathic DSPS.
The aim of this literature study is to investigate the potential added value of DLMO in diagnosing DSPS in patients suspected for delayed sleep phase syndrome.

METHODS

Definitions
DLMO from saliva is measured at scheduled time points until the patient falls asleep. Melatonin onset is established at the moment when melatonin level increases above 4pg/mL and remains elevated through following sampling.[5]

Search strategy

A systematic literature search of Pubmed, EMBASE and CINAHL was performed on the 18th of March 2010. In order to find relevant original articles various synonyms within the determinant (e.g. DLMO-tests) and the outcome (e.g. DSPS) were combined. The determinant was defined by the DLMO test and synonyms of melatonin to include descriptions as 'measuring melatonin'. To minimise reporting and retrieving bias, the domain (i.e. patients suspected for DSPS) was excluded in the search syntax. Determinant and outcome were searched in title and abstract field. For the search in EMBASE 'Medline' was excluded, to avoid double search in Pubmed/Medline. After filtering doubles, title and abstract were screened using predetermined inclusion- and exclusion criteria shown in the flowchart (fig 1). Only original articles were included, not reviews. Of the remaining articles full-texts were retrieved, otherwise, authors were approached personally. Full texts of the available articles were studied, using also predetermined inclusion- and exclusion criteria (fig 1). The references of remaining articles were studied to identify missing relevant studies not identified by this search strategy.

Critical appraisal

The relevance, size of study population, study design, validity and statistics were critically appraised. A cross-sectional study design is acknowledged as the most desired one for diagnostic studies, because it compares a new test with a reference standard and shows the added value of the new test.[11,12]

RESULTS

The literature search (fig.1) yielded 2779 articles, after filtering doubles and screening on title and abstract 21 articles were extracted for full-text analysis. Of the 19 full-text articles available, 6 were relevant considering the clinical query. Unfortunately, two of them investigated DSPS with ADHD as co-morbidity[8,9] and were excluded. The remaining four were selected for critical appraisal (table 1).

All four studies reported significant phase delay of salivary melatonin secretion onset in DSPS patients compared to controls.[13-16] However, three out of four studies (Chang et al.[14], Wyatt et al.[15] and Nagtegaal et al.[16]) did not answer the clinical query completely.

In these studies melatonin rhythms and sleep schedule differences in DSPS patients were analysed without investigating a potential added value of the DLMO test to other diagnostic instruments. Therefore, these studies were considered as next best evidence.

The best evidence to answer the clinical question is the study of Rahman et al.[13] Rahman et al.[13] conducted a cross-sectional study on fifty-six participants suspected for DSPS. Sleep diary and polysomnography, were used.[2] Subsequently all participants underwent DLMO testing to evaluate the circadian rhythm delay. The potency of DLMO in diagnosing DSPS was compared to the reference standard. Based on the presented data, it was possible to calculate the positive predictive value (87.5%, CI 79%-96%) and negative predictive value (87.5%, CI 79%-96%) of the test together with pre- and post-test probability (55%, CI 42%-68%, and 87.5%, CI 79%-96%, respectively). The absolute added value of DLMO in diagnosing DSPS in young adults in this study was 32.5%.

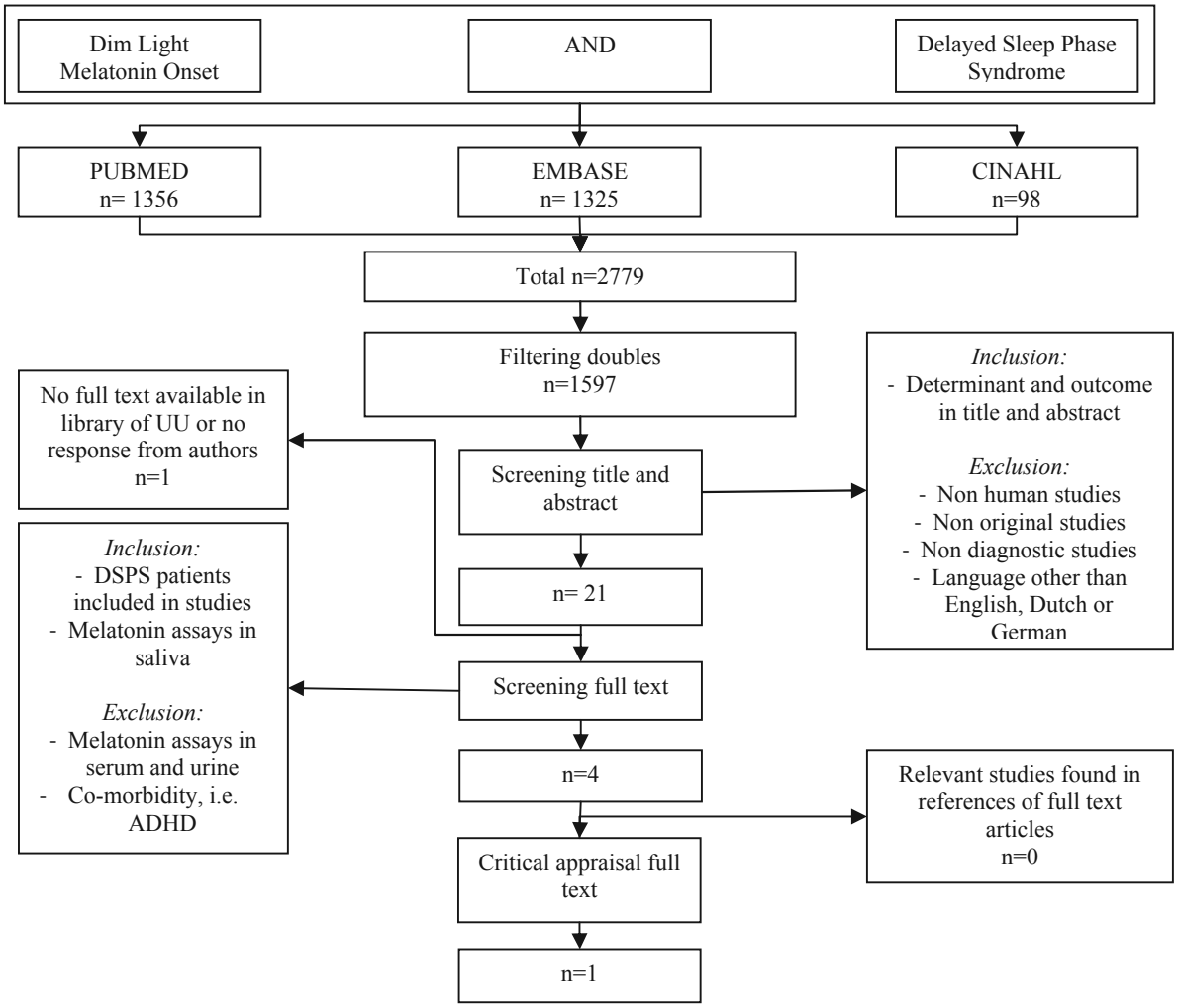

Figure 1. Flowchart

DISCUSSION

The aim of this literature study was to investigate the potential added value of DLMO in diagnosing DSPS. Only one article was able to answer the clinical query. Rahman et al.[13], showed 32.5% added value of DLMO above reference test for diagnosing DSPS in adults. In the remaining three case-control studies only a significant difference in melatonin onset time between DSPS patient and controls was demonstrated (Chang et al.[14], Wyatt et al.[15] and Nagtegaal et al.[16]). This only indicates a potential value of DLMO as a diagnostic test.
Of course, some limitations of this evidence based case report should be mentioned. Firstly, the fact that only one appropriate article was available. This could probably be explained by the facts that the presented subject is hardly examined or negative outcomes are less often published. The remaining article[13] presented a small amount of patients, which could lead to less representative results. Also the lack of outcome blinding in this article could lead to information bias. Secondly, Rahman et al.[13] used a polysomnography together with medical records as a reference standard. Polysomnography is not used routinely in the diagnosis of DSPS. Unsatisfactory, the added value of DLMO to patient characteristics and medical records only remains unknown. Thirdly, a more general limitation should be mentioned.

Table 1. Critical appraisal

Article	*Relevance*			*Population*	*Study design*[e]	*Validity*					*Statistics*		*Level of evidence*[m]
	Domain[a]	Determinant[b]	Outcome[c]	Number of patients with DSPS vs controls[d]		Blinding[f]	Standardisation[g]	Stratification[h]	Missing data (n)[i]	Transparancy of missing data[j]	Possibility to determine absolut difference[k]	Possibility to determine 95% CI[l]	
Rahman et al., 2009[13]	●	●	●	**56 vs 0**	**CS** ●	○	●	○	**0**	●	●	●	**8/10**
Wyatt et al., 2006[15]	●	●	●	**8 vs 8**	**CC** ○	○	●	○	**0**	●	○	○	**5/10**
Chang et al., 2009[14]	●	●	●	**66 vs 56**	**CC** ○	○	●	○	**105**	○	○	○	**4/10**
Nagtegaal et al., 1998[16]	◐	●	●	**3 vs 0**	**PS** ○	○	◐	**n/a**	**0**	●	○	○	**4/10**

[a]Domain: ● adults suspected for DSPS, ○ no suspicion; [b]Determinant: ● DLMO, ○ no DLMO; [c]Outcome: ● DSPS, ○ no DSPS; [d]Population: population size in numbers; [e]Study design: ● CS cross sectional, ○ CC case- control, ○ PS patient series; [f]Blinding (determinant and outcome): ● yes , ○ no; [g]Standardisation: ● yes, ◑ moderate, ○ no; [h]Stratification: : ● yes, ○ no, n/a not applicable; [i]Missing data: in numbers; [j]Transparancy of missing data: ●yes, ○ no; [k]Possibility to determine absolute difference: ● yes, ○ no; [l]Possibility to determine 95% CI: ● yes, ○ no; [m]Level of evidence: ● 1 point, ◑ ½ point ○ 0 points; Missing data and transparency counts for one point

A DLMO-test describes the moment of melatonin increase, but according to the ICSD-criteria it is still unclear how much later this rise must be seen in DSPS patients compared to controls. DSPS patients fall asleep typically after 2.00 am, and melatonin onset could be expected around this time, but this remains not evidence based.[2] Finally, in literature there is no cut off point determined above which an added value is sufficient. To analyse a diagnostic test a blinded cross-sectional study is recommended to evaluate test characteristics. After which a multivariate regression analyses should be performed to evaluate other risk factors, such as age, gender and comorbidity. Cost-effectiveness and clinical outcome in terms of co-morbidity should also be investigated.[11,12]

CONCLUSION

Only one study investigating the added value of DLMO in diagnosing DSPS was found after an extensive literature search. The added value above sleep diary combined with polysomnography was 32.5% in this study. To answer the question whether a DLMO-test has an added value in diagnosing DSPS to clinical history more research should be done. For

instance a cross-sectional study to compare DLMO to clinical history, in a larger study population, with multivariate regression and cost-effectiveness analyses.

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GRADUAL TERMINATION OF SHORT TERM MELATONIN TREATMENT IN CHILDREN WITH DELAYED DIM LIGHT MELATONIN ONSET: EFFECTS ON SLEEP, HEALTH, BEHAVIOR PROBLEMS, AND PARENTING

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INTRODUCTION

In the sleep-wake cycle melatonin plays a central role. Melatonin is a hormone that is produced in the pineal gland in the brains and increases sleep propensity. Melatonin secretion increases soon after the onset of darkness and is inhibited during the day. However, in some children melatonin secretion is delayed, resulting in problems falling asleep at an appropriate time. Exogenous melatonin administration, if well timed, can advance melatonin secretion.[1] Several studies found direct positive treatment effects on sleep onset and health or behavioral outcomes.[2,3] However, whether these effects continue after treatment discontinuation and what treatment duration is advisable, is still not clear.
Despite several studies reporting no adverse effects of long term melatonin use,[4,5] there is still a theoretical risk of delayed puberty onset. Besides, children should not be treated longer than necessary. Therefore, short term treatment is preferred above long term treatment. In this study the effects of a short term melatonin treatment were investigated. Since in a previous study positive effects of melatonin disappeared after immediate treatment discontinuation,[6] treatment was gradually discontinued in the present study. In addition, in this way it could be examined whether lower doses would suffice after melatonin use for a few weeks.
The main research question was: *What are the effects on chronobiotic and hypnotic sleeping behaviour, health, behaviour problems, parenting stress, and parenting in children with chronic sleep onset insomnia after three weeks treatment with melatonin and after two stop weeks with gradual discontinuation?* A second research question was: *Do behavior problems at baseline influence the effect of melatonin treatment?* Both subjective (sleep diaries and questionnaires) and objective (actometers, Dim Light Melatonin Onset (DLMO)) measures were used to answer these questions.

METHODS

Procedure and participants
The study was conducted in the Centre for Sleep-Wake disorders and Chronobiology in Hospital Gelderse Vallei in the Netherlands. Participants were 41 children between 5 and 12 years old (mean age 9.43), with chronic sleep onset problems and delayed melatonin secretion. Children were not eligible for participation if they were diagnosed with another sleep disorder (e.g., restless legs syndrome, narcolepsy, obstructive sleep apnea syndrome), if

the sleep onset problems were caused by medical problems (e.g., pain), blindness, or child-psychiatric or family problems, which could explain the sleep onset problems.
Treatment was started on the first Sunday after the appointment in the hospital. All children were instructed to start with a dose of 1 milligram at 19:00 h. If melatonin use did not have an effect, it was allowed to increase the dose with 1 milligram every four days to a maximum of 5 milligrams. Children took melatonin for three weeks. Then, treatment was gradually discontinued by first taking a half dose for one week and then stopping completely for another week. Parents filled in sleep diaries and children wore actometers during this whole period of five weeks. Other measures (questionnaires and DLMO) were obtained at baseline, directly after three weeks treatment and at the end of the stop week.

Measures
Chronobiotic sleeping behavior was measured by determining DLMO in the children's saliva. Children were instructed to chew on a cotton plug hourly from 19:00 to 23:00 hours in the evening by dim light. Children were not allowed to use melatonin in the evenings at which DLMO was measured.
Hypnotic sleeping behavior was measured with sleep diaries filled in daily by parents and with actometers. Actometers were used to obtain more objective information about sleep.
Health status of children was measured with the Functional Status II (FSII).[7] This is a 14-item questionnaire measuring the consequences of medical conditions for the child's behavior. Crohnbach's alpha was 0.57-0.82.
Behavior problems in children were measured with the Child Behavior Checklist (CBCL).[8] The CBCL is a comprehensive (112 items) questionnaire containing broad band scales and narrow band scales. With the broad band scales internalizing and externalizing behavior problems can be measured. In this study only total behaviour problems were addressed. As of the length of the scale, behavior problems were only measured at baseline and at the end of the stop week. Reliability was between .95 and .96.
Parenting stress was measured with the Nijmegen Parental Stress Index short version (NOSIK).[9] The NOSIK is a questionnaire with 17 items that measures to what extent parents experience stress in parenting their child. Reliability was between .93 and .95.
Parenting behavior was measured with the Nijmegen Parenting Questionnaire.[10] Three scales were used, namely affection-expression, responsiveness, and autonomy. Reliability for the total scale was between .86 and .91. In total there are 24 items.
All reliabilities were calculated for measurement occasions and parents apart.

Analyses
Data were analyzed using hierarchical linear models (linear mixed models) in SPSS, treating the repeated observations as nested within children. The different measurements were added as predictors in the models. For the questionnaires and DLMO, these were the different measurement occasions (i.e., at baseline, directly after three weeks treatment, and at the end of the stop week). For the sleep diary data, predictors are the data from different phases in the treatment (baseline, melatonin treatment, week with half dose, and stop week). This is the same for the actometer data, except that baseline data are not available.

RESULTS

First, it was determined which longitudinal structure described the variances and covariances best. Second, predictors were added and the fit of these models was compared to the fit of the models without predictors.

DLMO significantly decreased after treatment. This effect disappeared after treatment termination. Sleep latency measured with sleep diaries (time children spent in bed before falling asleep), was significantly longer at baseline and during stop week compared to treatment. The difference with the half dose treatment was not significant. When behavior problems measured at baseline were added to the model, the fit did not significantly improve (χ^2_D (1) = 3.840, p > .050). Behavior problems at baseline did not influence the effect of the melatonin treatment. For sleep latency measured with the actometers, it was also found that sleep latency was longer in the stop week. Actual sleep time was significantly less in the weeks with half dose and no treatment compared to treatment.

Parents reported a significant improvement in health of their child after treatment. This effect disappeared after termination. Child behavior problems significantly decreased after treatment termination. Parents reported experiencing less parenting stress after treatment. This effect remained after treatment was discontinued. However, melatonin treatment did not result in changes in parenting behavior. The model with predictors added was not significantly better than the empty model (χ^2_D (2) = 4.082, p = .130). See Table 1 for fixed effects, covariance structures, and results of the chi-square difference tests.

Table 1. Results of hierarchical linear models analyses

Dependent variable	*Fixed effects*[a]	*Covariance structure*	*β*	*s.e.*	*p*	χ^2_D *(df, p)*[b]
DLMO	M2[c]	AR1[e]	-87.901	11.632	< .001	37.014 (2, < .001)
	M3[d]		-5.237	10.880	.633	
Latency (sleep diary)	Baseline	AR1	81.019	4.288	< .001	372.609 (4, < .001)
	Treatment		-	-	-	
	Half dose		2.931	2.809	.298	
	Termination		33.158	3.039	< .001	
Latency (actometer)	Treatment	AR1	-	-	-	175.226 (3, < .001)
	Half dose		.404	2.384	.866	
	Termination		36.591	2.629	< .001	
Actual Sleep Time (actometer)	Treatment	AR1	-	-	-	41.074 (3, < .001)
	Half dose		-8.438	4.178	.044	
	Termination		-29.238	4.611	< .001	
Health	M2	DI[f]	1.818	.578	.002	10.528 (2, .005)
	M3		-.065	.610	.915	
Behavior	M2	DI	-11.178	1.908	< .001	29.741 (1, < .001)
Parenting Stress	M2	AR1	-3.081	.833	< .001	14.437 (2, .001)
	M3		-3.381	1.040	.001	

[a] Intercepts are left out due to space considerations
[b] Chi-square values indicate differences between models with predictors and empty models
[c] M2 = 2nd measurement, directly after treatment
[d] M3 = 3rd measurement, directly after the end of the stop week
[e] First-order autoregressive
[f] Diagonal

DISCUSSION AND CONCLUSIONS

In accordance with what is already known from the literature, this study found that melatonin decreased sleep latency and increased actual sleep time. An interesting finding is that for sleep latency this effect remained during half dose treatment. Behavior problems at baseline did not influence the effects of melatonin treatment.
Apart from the positive effects on sleep, melatonin positively influences health, behavior, and parenting stress. Whereas the effect on health disappeared after treatment discontinuation, it would be interesting to see whether the effects on behavior and parenting stress continue to exist after a longer time. A reversed placebo effect could account for disappearance of some of the positive effects in the stop week. These issues will be further investigated in a future study.

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SPIKE-WAVE DISCHARGES AND SLEEP-WAKE STATES IN ENTRAINED AND FREE-RUNNING CONDITIONS

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INTRODUCTION

Spike-wave discharges (SWD) are electroencephalographic manifestations of seizure activity in WAG/Rij rats, a well know, validated animal model of human absence epilepsy[1]. The occurrence of SWD is influenced by at least two different factors: a circadian factor, which organizes seizures into a 24-hour rhythm, and the state of vigilance, which, dependent on its nature, either enhances or inhibits the generation of SWD. The interdependence of the two factors is obvious: a minimal number of SWD is seen at the beginning of the light phase of the light-dark cycle (12:12 LD), when animals have the highest amount of deep slow-wave sleep. Contrary to deep sleep, light slow-wave sleep and passive wakefulness are the most favorable for the occurrence of SWD[2, 3].
The light-dark cycle is the strongest synchronizer (Zeitgeber) for the mammalian biological clock. Its absence alters parameters of any circadian rhythm. The most prominent change is the period length, which will deviate from 24 hours. Conditions of constant environmental light has been reported to influence many circadian rhythms such as motor activity, body temperature or sleep-wake cycle[4, 5].
The purpose of the present study was to investigate whether the strong relationship between the occurrence of SWD and sleep-wake states observed in the 12:12 light-dark regime persists in constant environmental condition (free-running condition, FR), which is known to change the properties of circadian rhythms, including the sleep-wake cycle.

METHODS

Seven adult, male WAG/Rij rats were used in the experiment. Cortical EEG electrodes were implanted under inhalation anesthesia. EEG and motility recordings were made in 12:12 LD (lights on at 7 am, light intensity: 60 Lux) and the FR condition of constant dim light (light intensity: 5 Lux).
Four representative hours were analyzed: the second and penultimate hour of the dark and the light phase of 12:12 LD and the corresponding hours of the active and the passive phase of the FR conditions. During the investigated hours, the amount of four sleep-wake states: active wakefulness (AW), passive wakefulness (PW), slow-wave sleep (SWS), and REM sleep (REM), as well as the number of SWD were counted. To study the relationship between the state of vigilance and the occurrence of SWD, every 5 sec. preceding and following each SWD were analyzed according to the procedure described previously[3]. Student t-tests for dependent variables were used to estimate statistically significant differences between the two experimental conditions (level of significance: $p<0.05$). All

experimental procedures were approved by the Animal Ethical Committee of the Radboud University Nijmegen.

RESULTS AND DISSCUSION

SWD were preceded mostly by PW and SWS, while AW and REM were the least probable states. There where no significant differences between LD 12:12 and FR. The percentage of PW was the highest among states of vigilance following the occurrence of SWD in both experimental conditions. This awakening effect has been reported previously in the study of Drinkenburg[3].In LD 12:12, AW was the second, whereas SWS and REM were the least probable states to follow SWD. In FR, AW was replaced by SWS, the amount of which appeared to be higher in comparison with LD 12:12. Results are presented in Fig. 1.

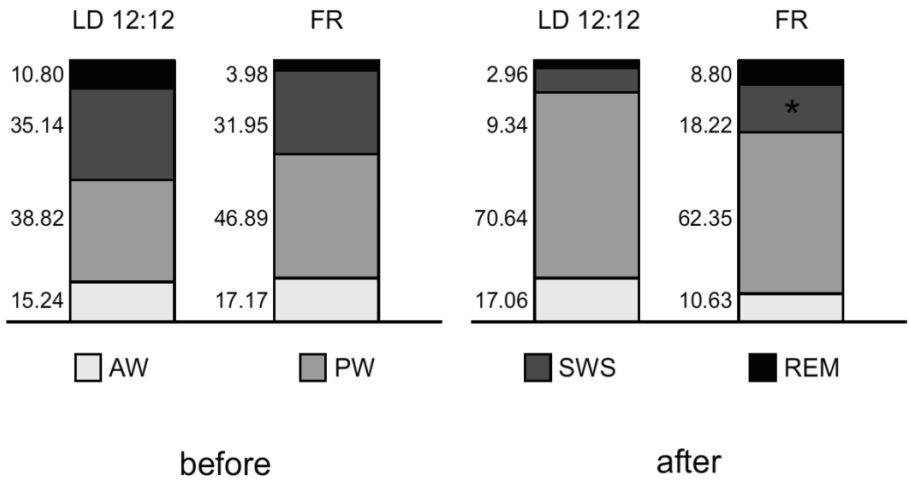

Figure 1. States of vigilance recorded before and after SWD in LD 12:12 and FR conditions. AW = active wakefulness, PW = passive wakefulness, SWS = slow-wave sleep, REM = REM sleep. In the FR, the amount of SWS following SWD was significantly higher, * $p<0.05$.

In 12:12 LD, the highest number of SWD was recorded at the beginning of the dark phase (mean ± SEM: 17.28 ± 3.43), after which a decrease was observed with a minimum at the beginning of the light phase (5.14 ± 1.50). At the end of that, the number of SWD increased, reaching similar level as at the beginning of the dark phase (13.88 ± 2.05).
In FR, both the maximum and minimum of the mean number of SWD were shifted. The maximum was recorded at the end of the passive phase (26.43 ± 5.49), while the minimum occurred at the end of the active phase (10.57 ± 4.35). There were no differences in the number of SWD between the two experimental conditions. Results are presented in Fig. 2.
Changes in the number of SWD across time, in both LD 12:12 and FR were found to be correlated with the course of two sleep-wake states: PW and SWS (Fig. 2). Positive correlations were found for PW ($r = 0.78$ in LD 12:12, $r = 0.25$ in FR): the changes over time of both PW and SWD showed a close correspondence. An increase in the amount of PW generally corresponded with the increase of the number of SWD (exception: the end of the passive phase in FR). Negative correlations were found for SWS ($r = -0.67$): changes in the amount of SWS always corresponded with opposite changes in the number of SWD. This correlation was also present in FR condition ($r = -0.81$), in which however, the course of PW and SWS seemed to be disrupted. The maximal amount of PW was shifted from the beginning of the dark phase of LD 12:12 to the beginning of the passive phase of FR. Also the minimum was changed from the beginning of the light phase of LD 12:12 to the end of

the active phase of FR. In the course of SWS, the maximum was shifted from the beginning of the light phase of LD 12:12 to the end of the active phase of FR (Fig. 2).

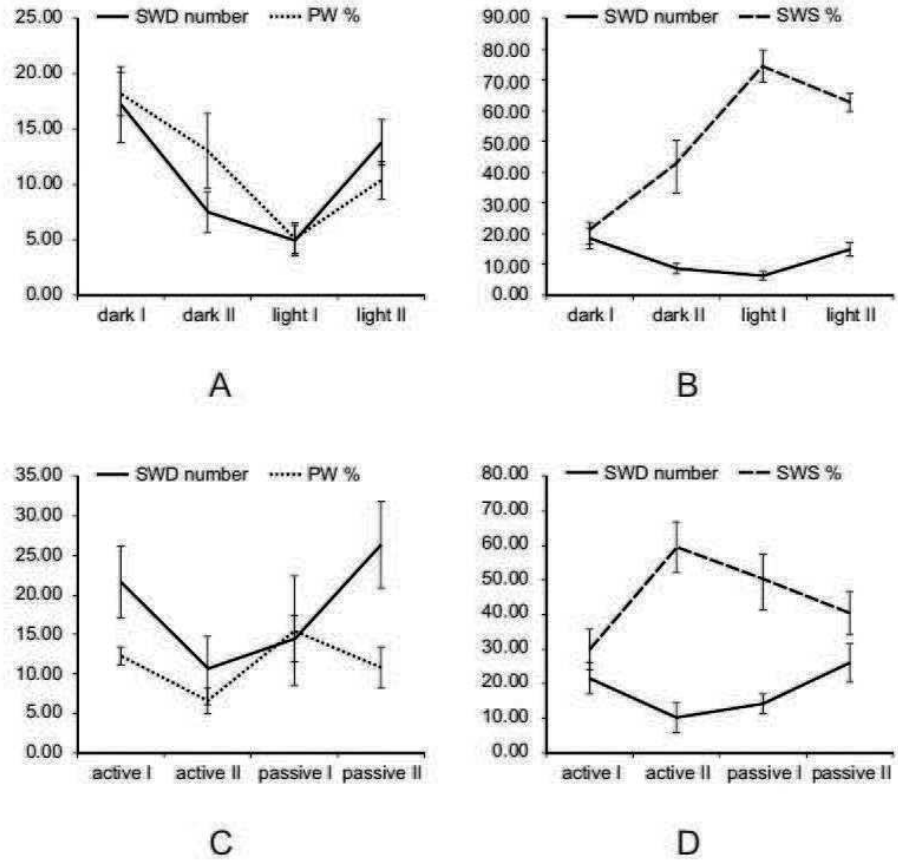

Figure 2. The course of the number of SWD, PW and SWS in LD 12:12 (A, B) and FR (C, D).

The preferred state for the occurrence of SWD is PW and SWS, which supports previous findings in WAG/Rij rats[3] and GAERS (another genetic animal model of absence epilepsy)[6] maintained on LD 12:12. The number of SWD correlated with the amount of PW and SWS. PW is highest at the beginning of the dark phase and decreases continuously to reach a minimum at the beginning of the light phase. At the end of that, an increase is observed. The course of SWS is opposite, with the minimum at the beginning of the dark phase and the maximum at the beginning of the light phase[7]. Our results confirmed these findings, however one should be aware that only 4 hours were analyzed. In the present study a distinction of SWS into light and deep was not made. However, the minimal number of SWD coincide with the beginning of the light phase, the time with the highest amount of deep SWS[8] .
The tendency of SWD to occur predominantly during PW and SWS was preserved in the FR. Similarly, the correlation between the amount of these sleep-wake states and the number of SWD was unchanged, despite the fact that some disturbances in the course of both PW and SWS were found. These alterations suggest that the entire distribution of sleep-wake states is shifted, which has been reported previously for rats maintained in constant dim light[4].
PW dominated among states of vigilance following the occurrence of SWD in both experimental conditions, which is in accordance with previous findings[3]. However, this awakening effect of SWD is diminished in FR. Our results suggests that the relationship between SWD and sleep-wake states following their occurrence, might be not so tight as between SWD and the state preceding their occurrence, thus more susceptible to circadian modulation.

CONCLUSIONS

The circadian factor plays a role in organizing the occurrence of SWD into a 24-hour rhythm. However, the relationship between SWD and sleep-wake states seems to be constant and independent from circadian modulation. SWD occur most frequently during passive wakefulness and light slow-wave sleep, regardless whether the circadian timing system is entrained to 12:12 LD or not. Moreover, alterations in the distribution of sleep-wake states caused by the constant environment do not influence this relationship. SWD always occur frequently at phases in which the amount of passive wakefulness is high and quite rare when deep slow-wave sleep dominates.

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CHEMOSENSITIVITY IN OBSTRUCTIVE SLEEP APNEA (OSA) ACCORDING TO GENDER AND EFFECT OF LONG-TERM CPAP THERAPY

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INTRODUCTION

Ventilatory control abnormalities, such as inappropriate levels of ventilation in response to hypercapnia, hypoxia, or resistive loading, have been postulated to contribute to the pathogenesis of disordered breathing during sleep[1-5]. In non-hypercapnic OSA patients CO_2 drive is slightly but significantly increased, and one year CPAP therapy may influence CO_2 sensitivity in these patients[6]. However, most patients evaluated in literature are male. Therefore, we wondered whether these findings could be attributed to female OSA patients as well. The present study evaluated the hypercapnic ventilatory response (HCVR) of women in the presence of snoring or OSA and also focused on the effect of long-term CPAP therapy on chemosensitivity.

METHODS

Comparative study (Descriptive study)
6 groups of patients were studied in basic conditions: male controls, female controls, male snorers, female snorers, normocapnic male OSA, normocapnic female OSA. The characteristics of these groups are summarised in Table 1. In the afternoon - before polysomnography - patients underwent the measurement of HCVR according to the Read method (SHCVR: slope; IHCVR: intercept)[7].

CPAP study
In a second part of the study (CPAP study) 42 patients with predominantly obstructive sleep apnea syndrome (AHI>20, age 55±1 y, BMI 33±1 kg/m^2, FEV_1 99±3 %pred) were treated with CPAP. Selected patients (during Night 1) were included into the following study protocol. The day before CPAP titration, patients underwent the assessment of HCVR. Patients then were treated chronically with CPAP at night with a fixed pressure. After at least one month and one year they were studied again with determination of arterial blood gases and HCVR.

RESULTS AND DISCUSSION

301 patients were evaluated (50±1 y; M/F 156/145; BMI 30±1 kg/m^2; weight 85±1 kg; height 169±1cm). In 42 OSA patients CO_2 drive was studied at baseline, after 1 M and after 1 y CPAP.

Comparative study

Patients characteristics are shown in Table 1. The breathing pattern of the several groups is depicted in Table 2. The slope of HCVR was significantly higher in male snorers as well as in male OSA, compared to female patients. Male OSA also had a higher SHCVR than male snorers. A statistically significant difference in the intercept was only present between the OSA groups (Table 3).

Table 1. Patients characteristics of the different groups

	Male controls	Female controls	Male Snorers	Female Snorers	Male OSA	Female OSA
N	11	9	59	60	86	76
Age (y)	40±3	40±4	46±1	50±1	52±1*$^{+}$	54±1*$^{+}$
BMI (kg/m²)	23±1	22±1	28±1	29±1*	31±1*$^{+}$	33±1*
$PaCO_2$ (mmHg)	42±1	39±2	40±0^{-}	38±1	41±0	39±0
PaO_2 (mmHg)	104±5	98±4	77±2*	89±1	75±1*	85±1^{X}
FEV_1 (% pred)	103±3	109±5	88±3	106±2^{+}	87±2*	103±2^{X}
AHI (#/h)	1±1	2±1	2±1	2±1	35±3*$^{+}$	37±3*$^{-}$

All data as mean±Standard Error

* p<0.05 compared to controls; $^{+}$ p<0.05 compared to male snorers; $^{-}$ p<0.05 compared to female snorers; ^{X} p<0.05 compared to male OSA

Table 2. Breathing pattern of the study groups

	Male controls	Female controls	Male Snorers	Female Snorers	Male OSA	Female OSA
AHI (#/h)	1±1	2±1	2±1	2±1	35±3 *$^{+}$	37±3 *$^{-}$
AI (#/h)	0±0	0±0	1±1	1±1	22±2 *$^{+}$	10±2 *$^{+X}$
OAI (#/h)	0±0	0±0	0±0	0±0	15±2 *$^{+}$	9±2 *$^{-}$
CAI (#/h)	0±0	0±0	0±0	0±0	7±1 *$^{+}$	1±0 ^{X}
HI (#/h)	1±1	1±1	1±1	1±1	13±2 *$^{+}$	26±2 *$^{+X}$

All data as mean±Standard Error; AI: apnea index; OAI: obstructive apnea index; CAI: central apnea index; HI: hypopnea index.

* p<0.05 compared to controls; $^{+}$ p<0.05 compared to male snorers; $^{-}$ p<0.05 compared to female snorers; ^{X} p<0.05 compared to male OSA

Table 3. Hypercapnic ventilatory response during wakefulness in all patients

	Male controls	Female controls	Male Snorers	Female Snorers	Male OSA	Female OSA
SHCVR (l/min/mmHg)	2.04±0.26	1.13±0.17	1.67±0.12	1.12±0.12 $^{+}$	2.2±0.11^{+}	1.60±0.06 ^{X}
IHCVR (mmHg)	42±2	44±7	37±2	36±1	40±1	31±2 ^{X}

All data as mean±Standard Error

$^{+}$ p<0.05 compared to male snorers; ^{X} p<0.05 compared to male OSA

CPAP study

42 patients met the inclusion criteria (Table 4). Mean objective CPAP compliance, based on built-in time counters, was 5.9±0.4 h/night after 1 year CPAP therapy. Results of HCVR and arterial gas exchange are shown in Table 5. In all patients CO_2 drive was high and accounted as a mean for 1.86 ± 0.16 l/min/mm Hg before treatment.

Table 4. Characteristics of OSA patients treated with CPAP

	All OSA	Male OSA	Female OSA
N	42	20	22
Age (y)	54±1	51±2	56±2 ^{X}
BMI (kg/m^2)	33±1	33±1	34±1
AHI (#/h)	47±3	46±5	48±4
$PaCO_2$ (mmHg)	41±1	42±1	40±1
PaO_2 (mmHg)	77±2	73±2	81±2 ^{X}
FEV_1 (%pred)	100±3	92±5	108±4 ^{X}

All data as mean±Standard Error
^{X} $p<0.05$, compared to male OSA

Overall, a significant decrease of SHCVR could be observed after 1 y CPAP therapy. SHCVR was significantly higher in male OSA at baseline ($p<0.01$) and could be normalised after 1 y CPAP. However, in female OSA CPAP did not influence SHCVR, although increase in PaO_2 occurred ($p=0.03$) with unchanged $PaCO_2$ ($p=0.51$). Also a small but statistically significant decrease in BMI could be observed in both study groups.

Table 5. Hypercapnic ventilatory response and arterial gas exchange at inclusion and during CPAP application

		Night 1	One month CPAP	One year CPAP
SHCVR (l/min/mmHg)	Overall	1.86±0.16	1.76±0.19	1.55±0.23 *
	Male OSA	2.37±0.22	2.21±0.23	1.79±0.18 *
	Female OSA	1.49±0.21	1.43±0.30	1.37±0.39
$PaCO_2$ (mmHg)	Overall	41±1	41±1	39±1 *+
	Male OSA	42±1	41±1	40±1
	Female OSA	40±1	40±1	39±1
PaO_2 (mmHg)	Overall	77±2	82±1	83±2 *
	Male OSA	73±2	77±2	79±2
	Female OSA	81±2	85±2	87±2 *
BMI (kg/m^2)	Overall	34±1	33±1*	32±1*$^{+}$
	Male OSA	34±1	33±1	32±1*
	Female OSA	34±1	33±1	32±1 *$^{+}$

All data as mean±Standard Error
* $p<0.05$ compared to Night 1; + $p<0.05$ compared to one month CPAP

The increased chemosensitivity in OSA is probably due to a progressive 'resetting' of the chemoreceptors (with higher sensitivity as a result), by a repetitive stimulation of these chemoreceptors [8,9]. Consequently, enchanced chemical drive will lead to more ventilatory oscillations during sleep,[10] and can lead to more severe OSA. Therefore, this enhanced SHCVR in male OSA is probably an explanation why OSA is more prevalent , but also more severe in male than in female. Moreover, our study demonstrated that chronic CPAP therapy

can decrease SHCVR in male OSA patients, parallelled by an improvement in arterial gas exchange. Since baseline SHCVR was only elevated in male OSA, CPAP will downregulate SHCVR exclusively in this patient category.

CONCLUSIONS

In snoring and OSA sex differences can influence CO_2 drive and these findings may support evidence for a contribution of respiratory control to the pathogenesis of OSA. Long-term 1-y CPAP therapy can reset and downregulate CO_2 drive to normal values in OSA, but only in case of male gender. Therefore, other factors must be involved as well to explain the pathophysiology of OSA in women.

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PREVALENCE OF COMPLEX SLEEP APNEA (COMPSAS) AND CLINICAL AND POLYSOMNOGRAPHIC CHARACTERISTICS

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INTRODUCTION

Last years CompSAS has been proposed as a prevalent disorder, presenting during CPAP titration in patients with predominantly obstructive sleep apnea hypopnea [1-10]. Prevalence data differ between 2.5% and 15%. Most studies are however performed in the USA, Japan and Australia. Large European studies are however lacking. Moreover, it could be questioned whether CompSAS is a real clinical entity or just a polysomnographic observation [7,8]. Previous studies reported a higher prevalence in male OSA, a lower BMI, and/or a higher incidence of ischemic heart disease and chronic heart failure [2,4,6]. Others suggest that these central apneas are provoked due to application of inappropriately high CPAP pressures. Conclusions are however speculative [7,8]. Besides this, CompSAS is equivalent to CPAP emergent sleep apneas, and hence, to disturbed sleep and persisting complaints. As a consequence, this can lead to more CPAP failure, lower CAP compliance, repeated sleep studies, unchanged morbidity and mortality during CPAP therapy, hence to an increased burden to the patient. Therefore, CompSAS is a real challenge in sleep medicine. The aim of the present study is to evaluate the prevalence of CompSAS during routine manual CPAP titration, in a clinical sleep disorders centre and to describe the clinical characteristics of CompSAS patients, compared to OSA patients with a normal response during CPAP titration.

METHODS

Prevalence study

392 CPAP titration nights during polysomnography, obtained between 1991 and 1998, were retrospectively evaluated. 50 studies performed in patients with predominantly central sleep apnea hypopnea (AHI>20, CAI>OAI) were excluded from analysis. CompSAS was considered if during CPAP titration: OAI decreased, while CAI increased (≥5), with predominantly central apneas (>50% of AI)[2]. OSA patients who demonstrated a normal response during CPAP titration were defined as 'normal' OSA. In our routine titration procedure CPAP pressure is only carefully elevated when central apneas develop.

Clinical and polysomnographic evaluation

In addition, the patients files of all CPAP titrations between 1991 and 2009 were screened for CompSAS. Clinical and polysomnographic data in these CompSAS patients were obtained from our routine sleep questionnaire (which enquired about the symptoms of disturbed sleep), physical examination, arterial blood gas analysis, lung function testing and polysomnography. Symptom severity were graded by the patients from 0 (absent) to higher

values (severe) [e.g. snoring from 0 to 4, excessive daytime sleepiness from 0 to 3, falling asleep during the daytime from 0 to 2, memory losses from 0 to 1, alertness in the morning from 0 to 1, and so on]. The characteristics of CompSAS patients were compared with data obtained in 'normal' OSA patients from the prevalence study.

RESULTS AND DISCUSSION

Prevalence study

349 CPAP titrations were evaluated (262 patients with predominantly OSA and 87 patients with sleep hypopnea syndrome)(Table 1). When this group was evaluated overall, only 9 patients demonstrated a CompSAS pattern (2.6%). Optimal pressure was determined at 7±1 mbar. Comparison between 'normal' OSA and CompSAS did not show any significant difference concerning age, BMI, arterial blood gases, routine lung function pattern, severity of hypoxemia, AHI or alveolo-arterial oxygen gradient. Due to the low number of CompSAS patients, a reliable comparison could not be made. In 303 'normal' OSA a complete dataset was available (including sleep questionnaire and physical examination as well), which was used for the clinical and polysomnographic comparison study.

Table 1. Patients characteristics of "normal" OSA and CompSAS patients (prevalence study).

	'Normal' OSA	*CompSAS*
N	340	9
M/F	297/43	9/0
Age (y)	51±11	52±9
BMI (kg/m^2)	32±6	33±9
$PaCO_2$ (mmHg)	39±4	37±2
PaO_2 (mmg)	78±11	82±12
FEV_1 (%pred)	92±20	87±13
FEV_1/VC	98±19	99±14
TLC (%pred)	100±13	100±13
AHI (#/h)	57±31	55±37
OAI (#/h)	23±25	31±23
CAI (#/h)	3±7	5±7
HI (#/h)	31±21	19±21
SaO_2<90% (min)	26±30	21±15
$MinSaO_2$ (%)	72±13	67±10
$AaDO_2$ (mmHg)	22±11	20±12

All data as Mean±SD; M/F: male/female ratio; BMI: body mass index; $PaCO_2$: partial arterial CO_2 pressure; PaO_2: partial arterial oxygen pressure; FEV_1: forced expiratory volume in one second; VC: vital capacity; FEV_1/VC: tiffenau ratio; AHI: apnea hypopnea index; OAI: obstructive apnea index; CAI: central apnea index; HI: hypopnea index; SaO_2<90%: time oxygen saturation less than 90%; $MinSaO_2$: minimal oxygen saturation value; $AaDO_2$: alveolo-arterial oxygen difference;

Clinical and polysomnographic comparison study

In addition, after screening the patients files of all CPAP titrations between 1991 and 2008, complete data were obtained from 41 CompSAS (out of ±3500 CPAP titrations, 1.17%). The characteristics of these 41 CompSAS patients were compared with the previously obtained data in 303 'normal' OSA patients. Mean symptoms scores are shown in Table 2. Clinical and polysomnographic data are presented in Table 3. Comparison between 'normal' OSA and CompSAS did not show any significant difference concerning age, BMI, gender, or symptom severity (except for concentration problems and self reported awakenings).

Table 2. Symptoms severity in a large series of CompSAS and 'normal' OSA patients

	'Normal' OSA	*CompSAS*
N	303	41
M/F	268/34	38/3
Snoring	3.6±0.8	3.4±0.8
Hypersomnolence	1.3±0.8	1.3±0.8
Falling asleep during the daytime	1.9±1.2	1.7±1.2
Matinal headache	0.5±0.9	0.4±0.8
Epworth Sleepiness Scale	12±6	12±5
Memory disturbance	0.4±0.7	0.4±0.6
Concentration problems	0.3±0.5	0.6±0.5 *
Awakenings	1.1±0.9	1.6±1.3 *
Refreshed in the morning	0.6±1.3	0.4±0.5
Superficial sleep	0.3±0.8	0.5±0.5
Witnessed apneas	0.8±0.6	0.9±0.3
Nocturnal dyspnea or gasping	0.7±1.1	0.6±1.0
Paresthesias	0.2±0.6	0.3±0.5
Nocturnal myoclonus	0.3±0.9	0.4±0.5
Nocturia	1.3±1.4	1.5±1.1
Ethanol use in the past (# units a week)	17±28	11±15
Gastro esophageal reflux	0.6±0.9	0.7±0.9
Ethanol use at present (# units a week)	10±17	11±13
Tobacco use (# of packyears)	13±20	22±23 *

* $p<0.05$, compared to 'normal' OSA; all data as Mean±SD.

Table 3. Clinical and polysomnographic data in a large series of CompSAS and 'normal' OSA patients

	'Normal' OSA	*CompSAS*
Age (yrs)	51±11	53±9
BMI (kg/m^2)	32.3±6.5	30.6±6.2
Weight (kg)	97±20	96±23
Length (cm)	173±9	177±8 *
Established arterial hypertension	48/303 (15.8%)	10/41 (24.4%)
Nasal obstruction	106/303 (35%)	9/41 (22%)
$PaCO_2$ (mmHg)	39±4	40±4
PaO_2 (mmHg)	78±11	84±12 *
SaO_2 (%)	95±2	97±1 *
Systolic blood pressure (mmHg)	139±23	130±16
Diastolic blood pressure (mmHg)	90±16	80±14 *
VC (%pred)	94±16	101±15 *
FEV_1 (%pred)	92±20	101±16 *
SEI (%Time in Bed)	67±17	75±14 *
Stage Wake (% Time in Bed)	33±17	25±14 *
NREM sleep (% Time in Bed)	56±15	62±11 *
REM sleep (% Time in Bed)	10±6	12±7
Awakenings (#)	96±91	285±139 *
AHI (#/h)	57±31	51±27

* $p<0.05$ compared to 'normal' OSA

CompSAS patients were heavier smokers, were taller, had more concentration problems, had a higher PaO_2 and SaO_2, and had a lower diastolic blood pressure. A history of arterial hypertension was prevalent in both groups, while only 1 CompSAS patient had undergone CABG. Intake of antihypertensives, and nasal obstruction were equally distributed in both

groups. VC and FEV_1 were also significantly higher in CompSAS. The polysomnographic data showed less wakefulness, but more awakenings in CompSAS. CompSAS patients presented with similar degrees of severity, based on the nocturnal hypoxemia time and AHI. These results demonstrate that CompSAS occurs less often than reported in the literature, under the condition that CPAP therapy is started during manual CPAP titration. This could be an argument to support the hypothesis that CompSAS is induced by the application of inappropriate high pressures. Careful CPAP titration could therefore prevent the development of CompSAS and hence, CPAP failure. Moreover, these data also indicate that CompSAS is rather a minor problem. CompSAS patients can also demonstrate some statistically significant differences in physical characteristics, arterial blood gases, and lung function values, but its clinical significance is uncertain. However, CompSAS patients showed more awakenings during polysomnography, which was also perceived by the patients. This could argue for a predisposition of some OSA patients to develop CompSAS and against the statement that CompSAS is a completely iatrogenic disorder. Our study is the first one to report a longer smoking history in CompSAS, which is contradicting with a previous study [6]. Smoking can lead to inflammatory changes in the upper airway mucosal layers, and hence, to altered reflex activity.

CONCLUSIONS

CompSAS evaluated in our OSA population is less prevalent than reported in the literature so far. This could be related to a more careful CPAP titration procedure. CompSAS patients suffer more from awakenings at night, which could make them more vulnerable to develop central apneas.

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DEGREE OF SLEEPINESS AND CARDIAC ALTERATIONS IN OBSTRUCTIVE SLEEP APNEA (OSA) PATIENTS.

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INTRODUCTION

Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder associated with increased risk of cardiovascular morbidity and mortality (1). The OSA syndrome is characterized by repeated partial or complete closure of the pharynx, gasping episodes, sleep fragmentation and variable degrees of excessive daytime sleepiness (EDS). EDS is a critical symptom of OSA, but it is probably not pathognomonic (2). Moreover, it has been recognized as an important public health problem, estimated to affect as much as 12% of the general adult population and has an impact on several important factors, e.g. the quality of life, motor-vehicle and work-related accidents as well as impaired social functioning. There is however a variable expression of EDS in OSA patients and it does not correlate with the apnea hypopnea index (AHI). Prospective studies have shown the beneficial effects of continuous positive airway pressure therapy (CPAP), with a reduction in cardiovascular morbidity - arterial hypertension, and diastolic dysfunction, pulmonary hypertension - and mortality in OSA (3). Recent studies have shown however that CPAP was not able to control hypertension in non sleepy hypertensive OSA patients (4-5). This suggests that the symptom of self-reported EDS may identify a subset of individuals at greater risk of cardiovascular sequelae. The aim of this study was to assess associations between sleepiness and excessive cardiovascular alterations in OSA.

METHODS

Study population:

Seventy seven patients with moderate to severe OSA (AHI>20) and fourteen controls (AHI<15), were included in this study. OSA was confirmed by a full in hospital polysomnography (Multidisciplinary Sleep Disorders Centre, Antwerp University Hospital, Belgium) We defined three groups based on the degree of sleepiness assessed by the Epworth sleepiness scale (ESS): Gr 1: ESS≥10, sleepy ; Gr 2: ESS<10, non sleepy; Gr 3: controls. All patients had a variable degree of daytime sleepiness and were without confounding factors for sleepiness (diabetes, metabolic syndrome, depression) (Patient characteristics are shown in table 1). Physical examination and ECG were performed, as well as a complete 2-D and Doppler echocardiography.

Echocardiography:

Imaging was performed using Philips iE33 (Eindhoven, Netherlands). A standard 2D-doppler, M-mode and Doppler echocardiography were performed for systolic and diastolic cardiac function as well as a tissue doppler Sm dipyridamole stress echocardiography. We evaluated heart rate (HR), blood pressure (BP), cardiac output (CO), stroke volume (SV),

interventricular septum thickness (IVS), left ventricular ejection fraction (LVEF), right ventricular systolic pressure (RVPsys), intima media thickness (IMT) and flow mediated dilatation (FMD) in all groups. Tissue Doppler mitral annular systolic velocity measurements (Sm) were performed in 26 OSA patients and 8 controls before and after stress. Stress is defined as an augmentation of heart beats/min caused by administration of dipyridamole. Blood samples were taken from all patients for the evaluation of their lipid profile.

Sleepiness:
All patients completed the Epworth sleepiness scale (ESS), to assess their daytime daily sleepiness (score 0-24).

RESULTS AND DISCUSSION

The clinical characteristics are presented in Table 1. Sleepy patients had a significantly higher BMI ($p=0.02$) (32 ± 5 kg/m²) than non sleepy patients (28± 5 kg/m²). Minimum oxygen saturation was significantly lower ($p<0.01$) in sleepy (73 ± 13%) and non sleepy (79 ± 11%) patients compared to controls (88 ± 4%). IVS was significantly thicker ($p<0.01$) in sleepy patients (1.24 ± 0.21 cm) compared to non sleepy (1.10 ± 0.21 cm) and control patients (1.05 ± 0.19 cm). The CO, LVEF, SV, RVPsys, Sm, total chol, HDL, LDL, TG were not significantly different between sleepy, non sleepy and controls patients (table 2). Post stress improvement in Sm was observed in 43% of the sleepy OSA patients, 64% of the non sleepy OSA and 75% of controls.

Table 1. Patients characteristics.

	Sleepy OSA (n=47)	Non sleepy OSA (n=30)	Controls (n=14)	P value
Age (years)	47 ± 10	49 ± 11	48 ± 9	NS
BMI (kg/m²)	32 ± 5*	28± 5	31 ± 10	0.02
AHI (#/h)	58 ± 3^{+}	44 ± 18^{-}	9 ± 3	<0.01
Mean sat (%)	92 ± 4	94 ± 2	96 ± 1	NS
Min sat (%)	73 ± 13^{+}	79 ± 11^{-}	88 ± 4	<0.01
ESS	15 ± 4^{*+}	6 ± 2^{-}	9 ± 4	<0.01
HR (bpm)	80 ± 11	78 ± 9	79 ± 9	NS
BPsys (mmHg)	123 ± 14	126 ± 15	122 ± 13	NS
BPdia (mmHg)	72 ± 12	71 ± 11	70 ± 10	NS
Chol totaal (mg/dL)	209 ± 42	197 ± 47	196 ± 26	NS
HDL (mg/dL)	49 ± 13	51 ± 15	57 ± 16	NS
LDL (mg/dL)	140 ± 42	133 ± 38	128 ± 34	NS
TG (mg/dL)	136 ± 75	125 ±69	96 ± 89	NS

* $p<0.05$ (sleepy OSA compared to non sleepy OSA)
$^{+}$ $p<0.05$ (sleepy OSA compared to control subjects)
$^{-}$ $p<0.05$ (non sleepy OSA compared to control subjects)

Table 2. Cardiovascular parameters of sleepy OSA, non sleepy OSA and control subjects.

	Sleepy OSA (n=47)	Non sleepy OSA (n=30)	Controls (n=14)	P value
CO (l/min)	4.4 ± 1.3	4.2 ± 0.8	4.6 ± 0.8	NS
SV (ml)	61.5 ± 16.3	60.5 ± 9.6	63.4 ± 11.3	NS
RVPsys (mmHg)	27.3 ± 5.2	28.3 ± 6.9	27.4 ± 2.6	NS
IVS (cm)	1.24 ± 0.21*+	1.10 ± 0.21	1.05 ± 0.19	<0.01
LVEF (%)	58.4 ± 6.1	57.1 ± 6.3	61.2 ± 7.4	NS
Sm (cm/sec)	10.0 ± 3.0	9.1 ± 2.3	9.5 ± 2.4	NS
FMD (%)	6.9 ± 2.2	6.8 ± 2.6	6.2 ± 1.8	NS
IMT (mm)	591 ± 97	625 ± 59	583 ± 122	NS

* $p<0.05$ (sleepy OSA compared to non sleepy OSA)
+ $p<0.05$ (sleepy OSA compared to control subjects)

It has been shown that BMI is associated with daytime sleepiness. Hence, the slightly higher BMI in the sleepy patients could probably explain the higher degree of sleepiness, as shown in epidemiological studies. However, since our complete study population was obese, this effect has to be interpreted with caution. Inclusion of a number of non obese sleepy OSA patients could be useful to control this aspect. Another drawback is the evaluation of sleepiness, based on ESS, which is controversial, since it is considered as a subjective scale, strongly influenced by the individuals ability for introspection. Additional evaluation with a multiple sleep latency test (MSLT) to confirm these conclusions could be recommended.
It has been proven that a significant correlation exists between the IVS and AHI. Despite our sleepy and non sleepy population did not differ in AHI, we found a significantly elevated IVS in sleepy OSA patients, indicating left ventricular hypertrophy (LVH). LVH has systematically been associated with arterial hypertension and is more clearly related with nocturnal hypertension than with daytime hypertension. The pathophysiological mechanisms have however to be unraveled.
43% of the sleepy patients has shown a improvement in LV kinetics post stress, compared to 64% and 75% of non sleepy and controls. This may implicate increased myocardial ischemia as underlying mechanism in sleepy OSA patients. During apnea the inspiratory efforts against the occluded pharynx cause abrupt reductions in the intrathoracic pressure, with enhancement of the veneous return, distension of the right ventricle and leftward shift of the interventricular septum causing a reduced filling of the left ventricle. Moreover, nocturnal pulmonary hypertension is present in all OSA patients, which consists of cyclic swings, associated with apneic episodes and increased RV dimensions. In our patients without clinically overt right heart failure, pulmonary disease or respiratory insufficiency, reduced systolic function occurred.

CONCLUSIONS

The study demonstrated that sleepy OSA patients have a significant thicker IVS and have only limited post stress improvement in left ventricular kinetics compared to non sleepy and

controls. These findings may implicate increased myocardial ischemia as the underlying mechanism in sleepy OSA.

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SLEEP-WAKE
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Abstracts

MODULATION OF GROUP II METABOTROPIC GLUTAMATE RECEPTOR (MGLU2) ELICITS COMMON CHANGES IN RAT AND MICE SLEEP-WAKE ARCHITECTURE.

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Introduction
Compiling pharmacological evidence implicates metabotropic glutamate $mGlu_2$ receptors in the regulation of emotional states and suggests positive modulators as a novel therapeutic approach of Anxiety/Depression and Schizophrenia.
Methods
We investigated subcutaneous effects of the metabotropic glutamate $mGlu_{2/3}$ agonist (LY354740) on sleep–wake architecture in rat. To confirm the specific effects on rapid eye movement (REM) sleep were mediated via metabotropic glutamate $mGlu_2$ receptors, we characterized the sleep–wake cycles in metabotropic glutamate $mGlu_2$ receptor deficient mice ($mGlu_2R^{-/-}$) and their arousal response to LY354740. We furthermore examined effects on sleep behavior in rats of the positive allosteric modulator, biphenyl-indanone A (BINA) alone and in combination with LY354740 at sub-effective doses. LY354740 (1, 3 and 10 mg/kg) dose-dependently suppressed REM sleep and prolonged its onset latency.
Results
Metabotropic glutamate $mGlu_2R^{-/-}$ and their wild type (WT) littermates exhibited similar spontaneous sleep–wake phenotype, while LY354740 (10 mg/kg) significantly affected REM sleep variables in WT but not in the mutant. In rats, BINA (1, 3, 10, 20, 40 mg/kg) dose-dependently suppressed REM sleep, lengthened its onset latency and slightly enhanced passive waking. Additionally, combined treatment elicited a synergistic action on REM sleep variables.
Conclusion
Our findings show common changes of REM sleep variables following modulation of metabotropic glutamate $mGlu_2$ receptor and support an active role of this receptor in the regulation of REM sleep. The synergistic action of BINA on LY354740's effects on sleep pattern implies that positive modulators would tune the endogenous glutamate tone suggesting potential benefit in the treatment of psychiatric disorders, in which REM sleep overdrive is manifested.

Eur J Pharmacol. 2009; 28; 603(1-3):62-72.

NEW LIGHT ON LEG MOVEMENTS WITHOUT RLS, IN INSOMNIA: COINCIDENCE OR USEFUL INFORMATION?

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Objectives. In many patients with unexplained insomnia, polysomnography (PSG) reveals excessive (periodic or at random) leg movements. Groupwise, these patients have no other features in common. Dopaminergic therapy may suppress the leg movements but does not treat the insomnia. Part of this large group of patients may have periodic leg movements with features similar to those in patients with insomnia due to restless legs syndrome (RLS). Recently, Ferri et al. introduced a new method for assessment of leg movements in sleep. In a small group of patients with unexplained insomnia a specific periodicity in leg movements and decrement of these movements over the night, were similar to these parameters in definite RLS patients. It was hypothesized that this subgroup of unexplained insomnia patients might benefit of dopamine-agonists as used in RLS. The aim of the study is enlargement of the small group that was previously studied and search for endorsement of the hypothesis on therapeutic possibilities.

Methods. N= 80 patients (42 females; median age: 51 years, range 18-88) who were negative on an extensive screening for the origin of their insomnia, were included if they showed leg movements with a mean of 15/hour of sleep. Leg movements were measured and assessed according to the AASM rules (2007); recording and scoring of the accompanying PSG was done using the same rules. The periodicity index (PI) as defined by Ferri et al. and the course of the (periodic) leg movements over the night were used as parameters. Both were compared to the characteristics of these parameters in definite RLS patients.

Results. The PI values were 0.93 for NREM, 0.72 in REM and 0.92 for total sleep. The intervals between LM's varied between 0.5 and 90 s (definition) with most interval durations ranging between 15 and 45s and a mean duration of 24.3 s. These values are similar to those found in a population of definite RLS patients. Sequences of at least 3 LM's occurred all over the night. Groupwise, these sequences occurred preferentially in the first 5 hours of the nocturnal sleep. This finding is similar to that seen in definite RLS patients.

Conclusions. In a large group of patients with unexplained insomnia but with LM's without RLS, we found profiles of the LMs similar to those in definite RLS patients. Follow-up of these patients during DOPA therapy will learn whether they react to this treatment as successfully as definite RLS patients.

CHRONIC SLEEP DISTURBANCE IMPAIRS GLUCOSE HOMEOSTASIS IN RATS

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Epidemiological studies have shown an association between short or disrupted sleep and an increased risk for metabolic disorders. To assess a possible causal relationship, we examined the effects of experimental sleep disturbance on glucose regulation in Wistar rats under controlled laboratory conditions.

Three groups of animals were used: a sleep restriction group (RS), a group subjected to moderate sleep disturbance without restriction of sleep time (DS), and a home cage control group. To establish changes in glucose regulation, animals were subjected to intravenous glucose tolerance tests (IVGTT) before and after 1 or 8 days of sleep restriction or disturbance.

Data show that both RS and DS reduce body weight without affecting food intake and also leads to hyperglycemia and decreased insulin levels during an IVGTT. Acute sleep disturbance also caused hyperglycemia during an IVGTT, yet, without affecting the insulin response.

In conclusion, our data reveal that disturbance of the regular sleep-wake rhythm has a marked effect on glucose homeostasis and body weight control. Sleep disturbance directly leads to glucose intolerance and hyperglycemia and, on the long term, to weight loss accompanied with reduced insulin responses. The data further suggest that a disturbance of the normal sleep pattern, even without restriction of total sleep time, is sufficient to affect glucose metabolism and body weight maintenance.

Barf RP, Meerlo P, Scheurink AJW. Chronic sleep disturbance impairs glucose homeostasis in rats. International Journal of Endocrinology, Epub ahead of print, 2010.

MODELING HUMAN SLEEP PROPENSITY

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The 2-process model, initially put forward by Borbély in 1982, describes sleep-wake behavior as regulated by the additive interaction of a circadian and a homeostatic process. The output of the model is dichotomous, either sleep or awake, and not a continuous function of sleep propensity (SP). The "distance" between the homeostatic component S and the circadian component C at a given time might be accepted as a continuous measure of SP, implying additive interaction of C and S. However, an additive interaction misses two abundantly described components of the sleep wake cycle, namely the afternoon nap (or performance dip) zone and the evening wake maintenance (or forbidden sleep) zone.

We propose two modifications of the 2-process model, to include also these daytime variations. First, the modified model is based on the interaction of two main sleep drives, one for Slow-wave- and one for REM sleep. While we keep process S, we have replaced the circadian double-threshold process C by a single circadian sleep drive R, derived from REM sleep. Second, comparison between different modes of action between the two regulating processes strongly suggests that a model with a multiplicative interaction between S and R optimally describes the known variations of human SP. Multiplicative interaction of S with R implies that the two processes may either magnify or dampen each other at a given time.

Under the condition of a normal phase and duration of nighttime sleep, our SxR model successfully displays four characteristics across 24 hours for SP: (a) a major peak at nighttime, (b) a secondary increase peaking post-noon, (c) a local minimum at sleep offset in the morning and (d) a second local minimum in the evening hours. Simulations with delayed or advanced night sleep times suggest that the magnitude of the post-noon SP depends on the phase of the preceding night sleep period. While post-noon SP attenuated or disappeared with phase delays of night sleep, phase advancing resulted in an increase of SP during daytime. In contrast, the evening local minimum of SP remained stable in all conditions.

We conclude that a simple, straightforward multiplication of the intensities of two sleep drives, one circadian and the other homeostatic, appears to be sufficient to model the major aspects of the SP variations across 24 hours. Furthermore it is conceptually very attractive that in our model the two main constituents of sleep, REM sleep and non-REM sleep, both contribute to SP.

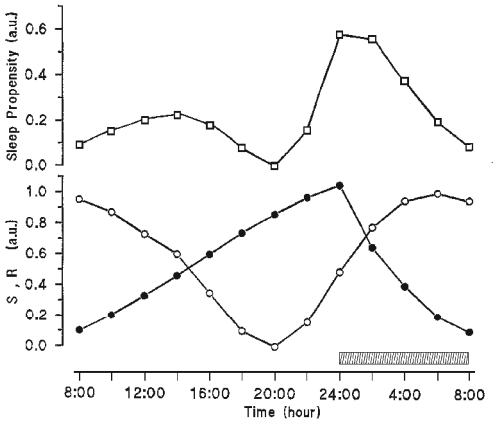

Figure 1. The time courses for the homeostatic sleep drive S (filled circles) and the circadian sleep drive R (open circles) are represented in the lower panel. The scale is relative, running in arbitrary units (a.u.) from 0 (low) to 1 (high). The sleep propensity function (SP, open squares, upper panel) was computed by multiplying the values of S and R at each point in time. An eight hour sleep episode (hatched bar) is assumed to take place between 24:00h and 08:00h. During this time S decays from a high initial value to a low level at the end of sleep.

SLEEP INFO SYSTEM (SIS) AUTOMATES WORKFLOW AND DATA HANDLING

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In the past twenty years, many new types of investigations have been introduced in the centre and the number of patients has increased strongly. Therefore, it became difficult to always have all patient data available at the right time and place, and to efficiently organize the work. For both purposes, the Sleep Information System (SIS) was developed by engineers working in the centre and collaborating intensively with medical and paramedical staff. Since 2009, SIS automates the workflow and data handling as follows.

The work is chained using task lists. A typical chain starts with a request from a family doctor, upon which a sleep physician orders investigations. Those are scheduled by the secretariat, carried out by technicians and psychologists, and analyzed. The physician judges the results and concludes the automatic letter to the family doctor. This letter and all appointments are communicated to the hospital information system (EZIS).

SIS retrieves patient identification data from EZIS, starts scheduled investigations by communicating with dedicated commercial software, archives the results and sends a full report of each patient, containing all investigations, to EZIS.

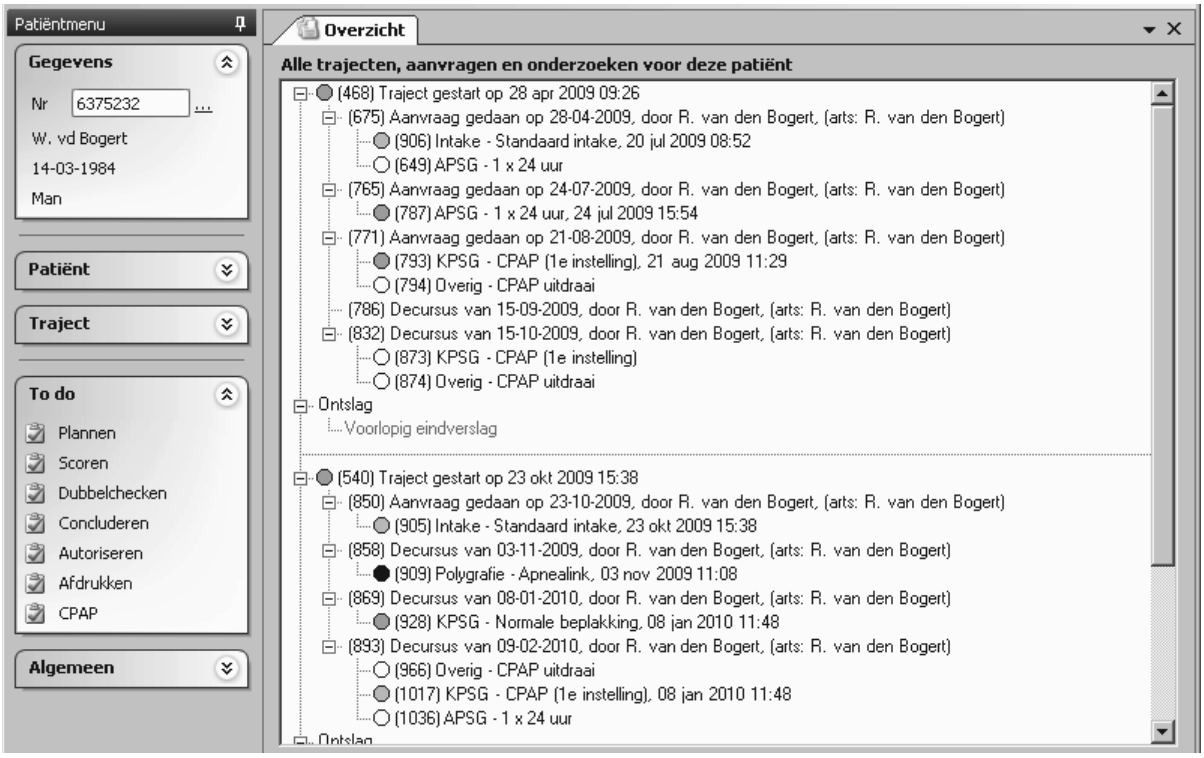

Figure. The core interface (in Dutch) lets the physician order and the technician start investigations, indicates their status by colours, and gives authorized users access to patient data and task lists.

SLEEP DISORDERS TEN YEARS AFTER BACTERIAL MENINGITIS,

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ABSTRACT

Introduction

Studies have shown that sleep problems occur after meningitis. We examined sleep disorders, sleep patterns and quality of sleep in patients with good recovery about 10 years after pneumococcal or meningococcal meningitis.

Methods

Patients were recruited from a nationwide prospective cohort of patients with good recovery, defined as Glasgow Outcome Scale score 5, after pneumococcal or meningococcal meningitis. Patients suffered from meningitis in 1998-2000. We included 17 patients after pneumococcal meningitis, 11 patients after meningococcal meningitis, and 13 control subjects (partners, siblings and close friends). Participants filled in the SF36 on quality of life (QOL), a questionnaire on subjective sleep quality (SSQ) for the present moment, the Epworth sleepiness scale on sleepiness and a sleep diagnosis list. They estimated their SSQ prior to the disease and filled in a sleep diary for a 2-week period recording naps during the day, sleep latency, sleep time, sleep efficiency, waking after sleep onset (WASO) and SSQ.

Results

The average time between meningitis and sleep evaluation was 109 months (SD=7). SSQ prior to the disease was comparable between groups (controls: 14.1 (SD=2.9) vs. patients: 14.6 (SD=3.3); P=0.60) but contrary to the expectation SSQ in the control group dropped significantly ten years after the disease (14.6 (SD=3.3) to 11.7 (SD=5.1); P=0.003) but not in the patient group. Data from the sleep diaries showed a similar pattern - although not significantly different between the groups - with shorter sleep latency and shorter WASO for the patient groups indicating better sleep. More sleep disorders were found in the patient groups (53%) than in the control group (23%), with a relatively high prevalence of circadian rhythm disorders in the group of meningococcal patients (46%), but these differences between the groups were not significant. QOL showed a significant decrease of pain of pneumococcal patients (51.2 (SD=10.1) vs. 81.4 (SD=18.4); P<0.001; higher score indicates less pain) and meningococcal patients (51.4 (SD=9.7) vs. 87.0 (SD=24.1); P=0.016).

Conclusion

Results indicate that sleep disorders may be more prevalent among patients, even with good recovery 10 years after pneumococcal or meningococcal meningitis. Subjective sleep quality however was better in patients, which may be due to improved quality of life 10 years after meningitis.

SKIN TEMPERATURE MANIPULATIONS AND SLEEP IN RATS

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In many mammalian species a relationship between body or brain temperature and changes in sleep and wakefulness has been established. However, experiments investigating a causal relationship between the two variables for a long time remained inconclusive. More recent experiments investigating the relationship between different skin temperatures, core body temperature and sleep in humans established a clearer relationship between sleep propensity and the time course of temperature in different regions of the body. The data particularly emphasized the importance of changes in proximal and distal skin temperature.
In the light of these new developments earlier research in rodents will be reviewed. In addition, new data investigating the effect of mild skin temperature manipulations on the occurrence and depth of sleep in the rat will be presented.
The data in the literature are compatible with the new insights in the relationship between different body temperatures and sleep. Our new data show that mild skin temperature manipulations are able to influence sleep in the rat during the rest phase, but not during the active phase. Increasing skin temperature promoted sleep by increasing non-rapid eye movement (NREM) sleep episode duration and REM sleep episode frequency, and decreasing waking episode durations.
It can be concluded that also in rodents, skin and body temperature changes are closely related to changes in vigilance states. The effect of skin temperature manipulations on sleep in the rat seems to be modulated by the circadian clock. Skin temperature manipulations clearly affect sleep in the rat, in a similar way as it influences sleep in humans.

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THE INFLUENCE OF SLEEP QUALITY, SLEEP DURATION AND SLEEPINESS ON SCHOOL PERFORMANCE IN CHILDREN AND ADOLESCENTS: A META-ANALYTIC REVIEW

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Introduction
Insufficient sleep, poor sleep quality and sleepiness are common problems in children and adolescents being related to learning, memory and school performance. It is still unclear whether or not these functions are mainly influenced by sleep quality, sleep duration, or sleepiness, which can be caused by sleep quality, sleep duration or a combination of these two sleep aspects. Due to methodological differences between studies, it is difficult to draw generalizable conclusions about the relationship between the different aspects of sleep and school performance. In order to gain more insight into these relationships three separate meta-analyses were conducted.
Method
The associations between sleep quality (k = 16 studies, N = 13,631), sleep duration (k = 17 studies, N = 15,199), sleepiness (k = 17, N = 19,530) and school performance were examined in three separate meta-analyses including influential factors (e.g., gender, age, parameter assessment) as moderators.
Results
All three sleep variables were significantly but modestly related to school performance. Sleepiness showed the strongest relation to school performance (r = 0.133; p < .001), followed by sleep quality (r = 0.096; p < .001) and sleep duration (r = 0.069; p < .001). Effect sizes were larger for studies including younger participants which can be explained by dramatic prefrontal cortex changes during (early) adolescence. Concerning the relationship between sleep duration and school performance age effects were even larger in studies that included more boys than in studies that included more girls, demonstrating the importance of differential pubertal development of boys and girls. No publication biases were present.
Conclusion
In summary, it can be concluded that all three sleep domains have a small, but significant effect on children's and adolescents' school performance. However, to be able to draw clear conclusions more research is needed, including experimental and longitudinal studies. Besides, it should be examined whether sleep might exert its influence via other variables, such as functioning at school. Such research can result in the development of programs aimed to improve school performance by changing children's and adolescents' sleep pattern.

Reference
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PULSE OXIMETER AVERAGING TIME; DEFINITION AND EFFECT ON THE CLASSIFICATION OF OBSTRUCTIVE SLEEP APNEA-HYPOPNEA SYNDROME (OSAHS)

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Introduction: In sleep medicine, pulse oximetry is used for monitoring oxygen saturation in patients with the obstructive sleep apnea-hypopnea syndrome (OSAHS). In fact, desaturations are an essential part of the AASM definition of a hypopnea. Several studies showed that the choice of pulse oximeter affects the outcome of the sleep study. In particular, the parameter averaging time; this parameter determines the duration of the smoothing filter implemented to minimize artifactual data. It has been shown that modifying averaging time results in underestimation of desaturation in the finger and the ear. However, the impact of averaging time on diagnosis (and consequently treatment) has never been studied. Therefore, the objective of this study was to determine whether the classification of OSAHS (normal, mild, moderate or severe) changes when pulse oximeter averaging time is doubled from 4 to 8 seconds averaging.

Methods: Cross sectional sleep study. The functional outcome measure was the oxygen-desaturation index (ODI), calculated using Nonin nVision®. ODI (desaturations/hour) was used to classify patients into the four severity categories of OSAHS. ODI of two pulse oximeters (Nonin®) with averaging times of 4 and 8 seconds were compared with a paired t-test and the Bland Altman method. Cohen's Kappa coefficient was used to test whether there was a difference in OSAHS classification. 18 patients participated in the study. Age and BMI were 48±9 and 31±4 respectively. All patients underwent in-home polygraphy for one night wearing both pulse oximeters. In addition, a mathematical model of pulse oximeter averaging was developed to simulate the effect of averaging on desaturations.

Results: ODI with 4 seconds averaging was 11% higher than ODI with 8 seconds averaging (paired t-test comparison, $p=0.03$). The Bland Altman plot revealed that ODI with longer averaging time was underestimated. No differences were found in the classification of OSAHS ($\kappa = 0.92$). With the mathematical model this could be explained by the fact that desaturations were often so large that the underestimated desaturation still met the desaturation criteria or that the desaturation time was long enough for the averaged signal to reach the minimum of the simulated desaturation.

Conclusion: In accordance with previous studies longer averaging times resulted in underestimation of desaturation. However, doubling pulse oximeter averaging time from 4 to 8 seconds did not affect OSAHS classification. This study implies that averaging time of current generation pulse oximeters does not affect diagnosis and treatment of OSAHS patients.

Abstract is presented at the ATS International Conference, New Orleans (May 14-19, 2010)

SLEEP DEPRIVATION IMPAIRS SPATIAL WORKING MEMORY AND REDUCES HIPPOCAMPAL AMPA RECEPTOR PHOSPHORYLATION

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Sleep is important for brain function and cognitive performance. Sleep deprivation (SD) may affect subsequent learning capacity and ability to form new memories, particularly in the case of hippocampus-dependent tasks. In the present study we examined whether SD for 6h or 12h during the normal resting phase prior to learning affects hippocampus-dependent working memory in mice. In addition, we determined effects of SD on hippocampal glutamate AMPA receptors and their regulatory pathways, which are crucially involved in working memory.

After 12h SD, but not yet after 6h, spatial working memory in a novel arm recognition task was significantly impaired. This deficit was not likely due to stress since corticosterone levels after SD were not significantly different between groups. In parallel with the change in cognitive function, we found that 12h SD significantly reduced hippocampal AMPA receptor phosphorylation at the GluR1 S845 site, which is important for incorporation of the receptors into the membrane. SD did not affect protein levels of cyclic-AMP dependent protein kinase (PKA) or phosphatase calcineurin (CaN), which regulate GluR1 phosphorylation. However, SD did reduce the expression of the scaffolding molecule A-kinase anchoring protein 150 (AKAP150), which binds and partly controls the actions of PKA and CaN.

In conclusion, a relatively short SD during the normal resting phase may affect spatial working memory in mice by reducing hippocampal AMPA receptor function through a change in AKAP150 levels. Together, these findings provide further insight into the possible mechanism of sleep deprivation-induced hippocampal dysfunction and memory impairment.

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Hagewoud R, Havekes R, Novati A, Keijser JN, Van der Zee EA, Meerlo P. Sleep deprivation impairs spatial working memory and reduces hippocampal AMPA receptor phosphorylation. Journal of Sleep Research 19: 280-288, 2010.

COPING WITH SLEEP DEPRIVATION: SHIFTS IN REGIONAL BRAIN ACTIVITY AND LEARNING STRATEGY

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Dissociable cognitive strategies are used for place navigation. Spatial strategies rely on the hippocampus, an area important for flexible integration of novel information. Response strategies are more rigid and involve the dorsal striatum. These memory systems can compensate for each other in case of temporal or permanent damage. Sleep deprivation (SD) has adverse effects on hippocampal function. However, whether the striatal memory system can compensate for SD-induced hippocampal impairments is unknown.

With a symmetrical maze paradigm for mice, we examined the effect of SD on learning the location of a food reward (training), and on learning that a previously non-rewarded arm was now rewarded (reversal training).

Five hours of SD after each daily training session did not affect performance during training. However, in contrast to controls, sleep-deprived mice avoided a hippocampus-dependent spatial strategy and preferentially used a striatum-dependent response strategy. In line with this, the training-induced increase in phosphorylation of the transcription factor cAMP response-element binding protein (CREB) shifted from hippocampus to dorsal striatum. Importantly, while sleep deprived mice performed well during training, performance during reversal training was attenuated, most likely due to rigidity of the striatal system they used.

Together, these findings suggest that the brain, when possible, compensates for negative effects of SD on the hippocampal memory system by promoting the use of a striatal memory system. However, effects of SD can still appear later on, because the alternative learning mechanisms and brain regions involved may result in reduced flexibility under conditions requiring adaptation of previously formed memories.

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Hagewoud R, Havekes R, Tiba P, Novati A, Hogenelst K, Weinreder P, Van der Zee EA, Meerlo P. Coping with sleep deprivation: shifts in regional brain activity and learning strategy. SLEEP, in press, 2010.

A TIME FOR LEARNING AND A TIME FOR SLEEP: THE EFFECTS OF SLEEP DEPRIVATION ON CONTEXTUAL FEAR CONDITIONING AT DIFFERENT TIMES OF DAY

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Sleep deprivation (SD) negatively affects memory consolidation, especially in case of hippocampus-dependent memories. Studies in rodents have shown that 5 h SD immediately following footshock exposure selectively impairs the formation of a contextual fear memory. In these studies both acquisition and subsequent SD were performed in the animals' main resting phase. However, in every day life, subjects most often learn during their active phase.

Here we examined the effects of SD on memory consolidation for contextual fear in rats when the task was performed at different times of the day, particularly, at the beginning of the resting phase or right before the onset of the active phase.

Results show that SD immediately following training affects consolidation of contextual fear, independent of time of training. However, in the resting phase memory consolidation was impaired by 6 h posttraining SD while in the active phase the impairment was only seen after 12 h SD. Since rats sleep at least twice as much during the resting phase compared to the active phase, these data suggest that the effect of SD depended on the amount of sleep that was lost. Also, control experiments show that effects of SD were not related to the amount of stimulation the animals received and were therefore not likely an indirect effect of the SD method.

These results support the notion that sleep immediately following acquisition, independent of time of day, promotes memory consolidation and that SD may disrupt this process depending on the amount of sleep that is lost.

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Hagewoud R, Whitcomb S, Heeringa AN, Havekes R, Koolhaas JM, Meerlo P. A time for learning and a time for sleep: the effect of sleep deprivation on contextual fear conditioning at different times of the day. SLEEP, in press, 2010.

HOW TO KEEP THE BRAIN AWAKE? THE COMPLEX MOLECULAR PHARMACOGENETICS OF WAKE PROMOTION

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Introduction
Wake-promoting drugs are widely used to treat excessive daytime sleepiness. The neuronal pathways involved in wake promotion are multiple and often not well characterized.
Methods
We tested d-amphetamine, modafinil, and YKP10A, a novel wake-promoting compound, in three inbred strains of mice.
Results
The wake duration induced by YKP10A and d-amphetamine depended similarly on genotype, whereas opposite strain differences were observed after modafinil. Electroencephalogram (EEG) analysis during drug-induced wakefulness revealed a transient approximately 2 Hz slowing of theta oscillations and an increase in beta-2 (20-35 Hz) activity only after YKP10A. Gamma activity (35-60 Hz) was induced by all drugs in a drug- and genotype-dependent manner. Brain transcriptome and clustering analyses indicated that the three drugs have both common and specific molecular signatures.
Conclusion
The correlation between specific EEG and gene-expression signatures suggests that the neuronal pathways activated to stay awake vary among drugs and genetic background.

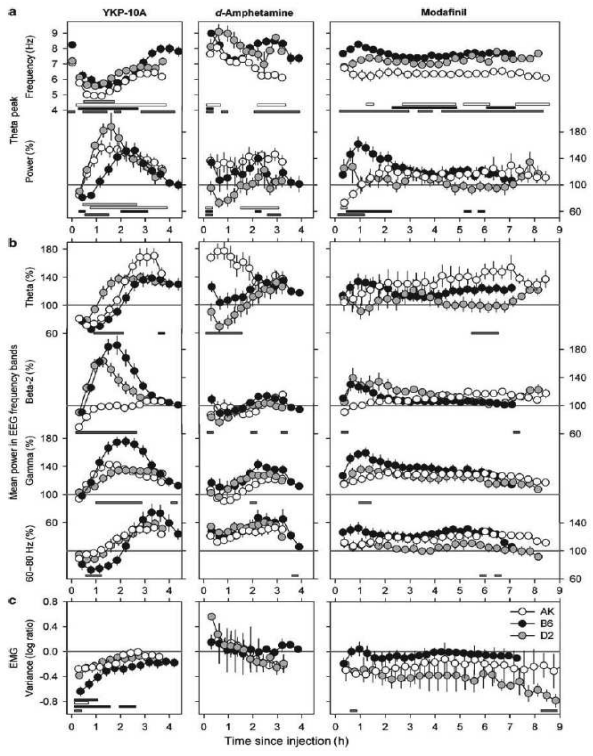

Figure: Time course of the effects of YKP10A, *d*-amphetamine, and modafinil on EEG theta-peak frequency and power (a), EEG power in selected EEG bands (b), and EMG variance during wakefulness between time of injection and sleep onset (c). (a) Theta-peak frequency (upper curve) after drug injection and after saline injection (first three dots plotted at time=0 h). Mean EEG power at theta peak was expressed as percentage of the theta peak power obtained after saline condition (=100%). (b) Mean EEG power in theta (upper), beta-2 (second), gamma, and 'high-frequency' (bottom curve) bands was expressed as percentage of baseline (for a and b, saline: n=27, 29, 30; YKP10A: n=15, 15, 15; *d*-amphetamine: n=6, 6, 8; modafinil: n=6, 8, 7; for D2, B6, and AK, respectively). (c) EMG variance was expressed relative (log ratio) to baseline (=0) (YKP10A: n=11, 15, 9; *d*-amphetamine: n=3, 2, 5; modafinil: n=3, 5, 3; for D2, B6, and AK, respectively). Gray, white, and black bars at the bottom indicate intervals in which theta-peak frequency differed from saline and EMG differed from baseline in D2, AK, and B6 mice, respectively (P<0.01; paired t-tests). Dark-gray bars mark intervals in which the drug effect differed according to genotype (P<0.01; one-way ANOVA). Open symbols: AK, black symbols: B6, gray symbols D2 mice.

Neuropsychopharmacology. 2009; 34(7):1625-40.

A NEW VIDEO ACTIGRAPHY METHOD FOR NON-CONTACT ANALYSIS OF BODY MOVEMENT DURING SLEEP

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Introduction. From body movements one can deduce sleep statistics such as total sleep time, sleep efficiency and fragmentation, and sleep/wake patterns. To assess sleep in the home situation, wrist actigraphy is often used. However, it is an on-body sensor which may influence sleep and it only collects data on the movement of one wrist. Video actigraphy methods overcome these issues. However, existing video actigraphy methods are computationally complex, need a high frame rate, a special type of camera with high resolution, and/or a specific viewing angle. We used a near-infrared (NIR) imaging technology to monitor the movements of a sleeping person. The proposed NIR video processing algorithm for motion detection and estimation is likely to outperform existing video actigraphy methods due to reduced computational complexity and fewer constraints. Privacy issues are limited due to real-time processing with volatile memory. As a first important step, we compared our off-body video actigraphy system with wrist actigraphy in a home setting.

Methods. Five participants were monitored using both wrist actigraphy and the video actigraphy system in their own bedroom. They were asked to install the system such that the bed was in line-of-sight and the non-visible NIR light was strong enough. The angle between image sensor and bed, type of bed and blankets varied among the participants. We compared the activity levels from wrist and video actigraphy.

Results. The motion data obtained by the video actigraphy system corresponded well to the wrist actigraphy signals for small, medium, and large motions. Arm, leg, head and torso movements, and tossing and turning, were detected even though the person was sleeping under a blanket and in various positions. The video system proved to be robust to different NIR lighting and installation settings and was easy to install.

Conclusion. Our off-body video actigraphy system can successfully replace on-body actigraphs to monitor a sleeping person's movements. The system is convenient and easy to use in real home testing situations. Importantly, it offers opportunities beyond the possibilities of wrist actigraphy, such as the motion analysis of specific body parts over longer periods of time relevant for e.g. PLMS detection. When developed further, the system may be a cheap and easy to use solution for personalized sleep awareness and for early and convenient diagnosis of sleep disorders.

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TIME-OF-DAY EFFECTS ON COGNITION IN PREADOLESCENTS: A TRAILS STUDY

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Introduction. Cognitive performance fluctuates during the day, due to variations in circadian arousal level. This study examined whether cognitive performance in school-aged children is affected by time-of-day, and whether these effects are more pronounced for cognitively more demanding tasks or task conditions.

Methods. Children, ages 10-12 yrs, were randomly assigned to a test session starting at either 8:30 (n=802), 10:00 (n=713), or 13:00 h (n=652). Speed and accuracy of information processing was assessed on tasks that assess input-related cognitive processes (e.g. detection, discrimination), central cognitive processes (e.g. serial search, sustained attention), and output-related processes (e.g. response organization and -inhibition), using the Amsterdam Neuropsychological Tasks program.

Results. The results of this study show that there are small (all effect sizes $\eta_p^2<.06$), but clear time-of-day effects on task performance in children. Sustained attention performance in the morning shows a speed-accuracy tradeoff with increased slowness and lapses, however, better feedback responsiveness and higher accuracy and perceptual sensitivity compared to the early afternoon. Performance on automatic, low-demanding processes is not influenced by time-of-day. However, increasing task complexity for working memory and visuospatial processing leads to higher performance decrements in the morning compared to the early afternoon.

Conclusions. Time-of-day effects in children were present in specific neurocognitive domains, such as attention and working memory, however, only under cognitively more demanding task conditions, or associated with time-on-task. In contrast to our hypotheses, circadian effects in performance were not found for all cognitively more loaded tasks. The findings indicate that evaluation of task performance in the clinical or educational setting should take into account potential time-of-day effects.

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RISK BEHAVIOR IN ADOLESCENTS INCREASES WITH SLEEP LOSS

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Introduction

During adolescence many hormonal, cognitive and social changes take place, increasing the biological need for sleep. However, a delay in sleep times and an increased social pressure induce chronic sleep loss during the school week, with a negative impact on cognitive behavior and mood. Risk behavior is part of the normal development in adolescence. Sleep loss may increase the occurrence of this behavior, especially during later adolescence, when a peak occurs in risk behavior. In this paper the relation is studied between adolescent sleep and risk behavior.

Methods

A questionnaire was administered to 37 boys and 61 girls (15-18 yrs) containing questions on sleep pattern, sleep behavior and sleep quality. The risk behavior questionnaire measured 6 types of behavior: criminal behavior, the use of drugs, unsafe sex, gambling, truancy and risky traffic behavior. To study the impact of personality factors like extraversion and neuroticism on risk behavior the Amsterdam Biographic Questionnaire was administered.

Results

All adolescents slept less than 9.2 hours and 18.9% slept shorter than 6:45 hrs. During the school week boys slept 34 minutes shorter than girls. 89.5% of the children needed more sleep than they got to feel fit.

A shorter total sleep time was significantly related with a higher average score on the risk behavior questionnaire (Spierman rho = -0.356, $p<0.001$). This relation was most obvious for criminal behavior ($p<0.01$), drug-use ($p<0.01$), gambling ($p<0.001$) and truancy ($p<0.05$).

A higher extraversion score was significantly related with a higher average score on the risk behavior questionnaire (Spierman rho = 0.307, $p<0.001$). This was mainly due to a higher score on the subscales drug-use ($p<0.01$), gambling ($p<0.05$) and risky traffic behavior ($p<0.001$). No significant relation was found between neuroticism and risk behavior.

A multiple regression model with risk behavior as dependent variable was significant ($R2 = 0.228$, $p<0.001$) and yielded gender, sleep quality and extraversion as most important predictors. Being an extravert boy with lower sleep quality seems to be more related with risk-raking behavior.

Conclusion

Evidence was found for a relation between sleep and risk behavior. An extravert personality is an extra factor triggering this behavior. Gender seems to be the most important factor for the development of risk behavior, leading to the conclusion that the relation between risky behavior and sleep is most prominent in boys

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EFFECTS OF AN EMOTIONAL FILM ON SLEEP EEG: RELATION WITH EMOTIONAL ATTENUATION OVER SLEEP

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Introduction

Many studies report a relation between sleep and emotion. Indeed, there is a high comorbidity of sleep disturbances with affective psychopathology, including anxiety and depression. REM sleep may be of particular relevance in this relation. For example, the majority of dream reports from REM sleep have an emotional content. Furthermore REM-sleep abnormalities are found in depression and changes in REM sleep percentages have been observed in relation to emotional recovery from traumatic life experiences. However, direct evidence for a role of sleep in emotional processing is scarce.
We have performed a controlled laboratory experiment to assess the relation between sleep phases and emotional coping directly.

Methods

A group of 38 subjects viewed emotionally neutral or distressing film fragments in the morning. During the ensuing 12-hour retention interval half of the subjects took a nap in the early afternoon; the other half in the late afternoon. During the nap EEG, EOG and EMG were recorded. The difference in circadian timing of the nap results in relatively more REM sleep during the early nap (203% more compared to late nap, $p = 0.07$) and more slow wave sleep (SWS) during the late nap (161% more compared to early nap, $p < 0.01$). In the evening subjects were reminded of the film through a series of stills. Emotional (Profile of Mood States) and physiological (GSR) arousal state were assessed at the beginning and end of the retention interval.

Results

Preliminary results show that reactivation of the emotional response towards the distressing film fragment is more strongly attenuated after the early nap than the late nap (significantly less anger, $p < 0.05$, and trends towards less tension, $p = 0.06$, and depression, $p = 0.19$). Moreover, there is an effect of the distressing film on sleep architecture with a trend level increase in REM sleep percentage ($p = 0.085$) and significant decrease in SWS percentage ($p < 0.05$) after the emotional film, compared to the neutral film.

Conclusion

The results suggest that emotionally distressing experiences tend to change sleep architecture, increasing REM sleep and depressing SWS. Furthermore, we have shown that the emotional response towards a distressing stimulus is especially reduced after a nap with a high REM content. The combined findings suggest a deleterious effect of distressing experiences on SWS and support a role of REM sleep in emotional coping.

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SUCCESS RATE OF SALIVARY DIM LIGHT MELATONIN ONSET MEASUREMENTS

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Introduction
Dim Light Melatonin Onset (DLMO) is the most reliable marker to assess circadian rhythmicity and to time administration of exogenous melatonin in the treatment of circadian rhythm disorders. At the Dutch national referral centre for circadian rhythm sleep disorders salivary DLMO is measured yearly in about 1,500 patients. Patients collect saliva conveniently in their home environment and send it to the laboratory for analysis of the endogenous melatonin concentration. The success rate of these salivary DLMO measurements in insomnia patients has not yet been studied and therefore we retrospectively analyzed the patients sample size of the year 2008. Furthermore, we studied the correlation between diary and PSG (polysomnography) sleep onset with DLMO to see if DLMO can be predicted by sleep onset time in the patients with information available from sleep diary or PSG.

Methods
Patients, who were referred to the sleep centre of the Gelderse Vallei Hospital, were asked to complete an online questionnaire. A Sleep Check containing saliva collections devices (Salivette®) was send to the patient in order to collect saliva at 5 consecutive hours for partial melatonin profiling. Melatonin concentration was measured with a radioimmunoassay (Buhlmann) and DLMO was defined as the time at which the melatonin concentration in saliva reaches 4 pg/mL.

Results
Out of 1,848 five point partial melatonin curves, DLMO was determined in 76.2% (n=1,408). DLMO significantly differed between different age groups and increased with age. Pearson correlations (r) between DLMO and sleep onset measured with PSG or a diary were (r= 0.514, p=<0.001, n= 54) and (r= 0.653, p=0.002, n= 20), respectively.

Conclusion
DLMO can be determined reliably by measuring melatonin in saliva that is conveniently collected at home. DLMO cannot be predicted reliably by sleep onset measured by PSG or diary.

Abstract will be presented at the ESRS 2010 Lisbon, Portugal.

THE INTERRELATIONSHIP BETWEEN SLEEP REGULATION AND THERMOREGULATION

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The circadian distribution of vigilance states and body temperature changes are tightly coupled. The increase in heat loss at the end of the day is associated with increased ease to fall asleep. Experimental data show that warming the skin or the brain can increase sleep propensity, sleep consolidation, and the duration of sleep. Anatomical and neurophysiological studies show that the pre-optic-anterior-hypothalamus (POAH) is the main integrator of sleep and thermoregulatory information. It integrates information on vigilance states, body temperature, and environmental temperature and influences vigilance states and body temperature in response. Animals that display daily torpor may be a valuable model to investigate the relationship between sleep and thermoregulation. During torpor these animals seem to apply similar strategies and physiological processes as humans during entrance into sleep, but in a more extreme way, providing an excellent opportunity to investigate these processes in more detail. More systematic investigations are needed to further our understanding of the relationship between sleep and thermoregulation, and may provide the basis to treat sleep disturbances with thermal strategies.

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COGNITIVE-BEHAVIORAL SELF-HELP TREATMENT FOR NIGHTMARES: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Introduction: Several cognitive-behavioral techniques are effective in reducing nightmare frequency, but the therapeutic factor (e.g. cognitive restructuring, systematic desensitization) remains unclear. The aim of this study was to compare the nightmare treatments imagery rehearsal therapy (IRT), exposure, and recording (keeping a diary) –in a self-help format– with a waiting list.

Methods: Participants were recruited through a Dutch nightmare website (www.nachtmerries.org). After completion of the baseline questionnaires, 399 participants were randomly assigned to a condition, received a 6-week self-help treatment (or were placed on the waiting list), and filled out the post-treatment measurements 11 weeks after baseline.

Results: Compared to the waiting list, IRT and exposure were effective in ameliorating nightmare frequency and distress, subjective sleep quality, anxiety (after imagery rehearsal), and depression (after exposure; $\Delta\ d = 0.25–0.56$). Compared to recording, IRT reduced nightmare frequency while exposure reduced nightmare distress ($\Delta\ d = 0.20–0.30$; $p < 0.05$). The recording condition was more effective compared to the waiting list in ameliorating nightmare frequency, nightmare distress, and subjective sleep quality ($\Delta\ d = 0.19–0.28$; $p < 0.05$). IRT had a more rapid reduction on the diary compared to exposure and recording.

Conclusion: IRT and exposure appear equally effective in ameliorating nightmare complaints. Exposure to nightmare imagery may function as the crucial therapeutic factor; however, cognitive restructuring may be a useful addition to increase immediate effects.

This study was funded by the Dutch Foundation for Mental Health, located in Amersfoort, the Netherlands (FPG20066126).

Lancee J, Spoormaker VI, van den Bout J. Cognitive behavioral self-help treatment for nightmares: a randomized controlled trial. Psychother. Psychosom. In press.

PROLONGED SLEEP RESTRICTION AFFECTS GLUCOSE METABOLISM IN HEALTHY YOUNG MEN

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Introduction. Sufficient sleep is a key component in the regulation of energy metabolism. Several epidemiological studies have shown that habitual short sleep duration is correlated with an increased risk of developing obesity and diabetes. In the present study, we simulated accumulating sleep restriction during five working days followed by two days of weekend recovery sleep and measured the changes in several metabolic parameters that occurred during this period, including glucose metabolism, serum leptin concentrations, and feelings of satiety.

Methods. Twenty-three healthy young men were randomly allocated to a control group (CON) or an experimental group (EXP). After two nights of 8 h in bed (baseline, BL), EXP spent 4 h in bed for five days (sleep restriction, SR), followed by two nights of 8 h (recovery, REC). CON spent 8 h in bed throughout the study. Blood samples were taken at 07:30 h after the BL, SR, and REC period and analyzed for glucose, insulin, IGF-1, and leptin.

Results. In EXP, insulin and insulin-to-glucose ratio increased after SR (159.9% and 160.8% of BL levels, respectively). IGF-1 levels increased after REC (111.7% of BL levels). Leptin levels were elevated after both SR and REC (163.3% and 123.1% of BL levels, respectively), while subjective satiety remained unaffected. No changes were observed in CON.

Conclusion. Prolonged sleep restriction – mimicking a working week – changes glucose metabolism and may lead to an increased risk of developing type 2 diabetes. In addition, sleep restriction does not affect hunger feelings and results in elevated leptin levels, suggesting that sleep restriction *per se* may not increase the risk of developing obesity. Hence, the previously observed epidemiological association between short sleep and obesity might be due to a common underlying factor rather than to a direct causation.

This study was supported by the EU Framework 6 (MCRTN-CT-2004-512362) and the Finnish Work Environment Fund (FWEF; 104073 and 108203).

Wessel M. A. van Leeuwen, Christer Hublin, Mikael Sallinen, Mikko Härmä, Ari Hirvonen, and Tarja Porkka-Heiskanen, "Prolonged Sleep Restriction Affects Glucose Metabolism in Healthy Young Men," International Journal of Endocrinology, vol. 2010, Article ID 108641, 7 pages, 2010. doi:10.1155/2010/108641

LONGITUDINAL RELATIONS BETWEEN SLEEP QUALITY, TIME IN BED AND ADOLESCENT PROBLEM BEHAVIOUR

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Background: This study aimed at investigating the unique and combined influence of sleep quality, time in bed, gender, and age at baseline on various adolescent problem behaviours over a two-year period. Additionally, effects of problem behaviour on sleep were examined.

Methods: The Youth Self-Report was administered to measure aggressive, delinquent, anxious/depressed, and withdrawn behaviour and somatic complaints. Sleep variables were assessed by a self-report questionnaire concerning sleep quality and time in bed. At the first measurement 650 adolescents participated in the study (328 boys and 322 girls; mean age = 13.36 years; age range 12 -15 years).

Results: Linear Mixed Model Analyses showed that sleep quality had a unique effect on all problem behaviours for both genders. Time in bed was directly related to externalizing behaviours. A time in bed below the mean time appeared to be a risk for boys' internalizing problem behaviour over time. With respect to girls, a shorter baseline time in bed was related to a faster increase of girl's anxious/depressed behavior and longer time in bed to a delayed decrease of withdrawn behavior and somatic complaints. The test of reversed effects showed less evidence of internalizing problem behaviour as predictor of time in bed. With regard to sleep quality, distinct relations with all problem behaviours were found.

Conclusions: Based on the results of this study it can be concluded that in addition to poor sleep quality, a short time in bed also contributes to the development of adolescents' problem behavior. A short time in bed appears to affect boys' internalizing problem behavior in particular. Problem behavior in turn does not predict time in bed. Sleep education and treatment of adolescent sleep problems are recommended as a prevention of problem behavior.

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THE ACOUSTICS OF SNORING

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Snoring is a prevalent disorder affecting 20–40% of the general population. The mechanism of snoring is vibration of anatomical structures in the pharyngeal airway. Flutter of the soft palate accounts for the harsh aspect of the snoring sound. Natural or drug-induced sleep is required for its appearance. Snoring is subject to many influences such as body position, sleep stage, route of breathing and the presence or absence of sleep-disordered breathing. Its presentation may be variable within or between nights. While snoring is generally perceived as a social nuisance, rating of its noisiness is subjective and, therefore, inconsistent. Objective assessment of snoring is important to evaluate the effect of treatment interventions. Moreover, snoring carries information relating to the site and degree of obstruction of the upper airway. If evidence for monolevel snoring at the site of the soft palate is provided, the patient may benefit from palatal surgery. These considerations have inspired researchers to scrutinize the acoustic characteristics of snoring events. Similarly to speech, snoring is produced in the vocal tract. Because of this analogy, existing techniques for speech analysis have been applied to evaluate snoring sounds. It appears that the pitch of the snoring sound is in the low-frequency range (<500 Hz) and corresponds to a fundamental frequency with associated harmonics. The pitch of snoring is determined by vibration of the soft palate, while nonpalatal snoring is more ‘noise-like’, and has scattered energy content in the higher spectral sub-bands (>500 Hz). To evaluate acoustic properties of snoring, sleep nasendoscopy is often performed. Recent evidence suggests that the acoustic quality of snoring is markedly different in drug-induced sleep as compared with natural sleep. Most often, palatal surgery alters sound characteristics of snoring, but is no cure for this disorder. It is uncertain whether the perceived improvement after palatal surgery, as judged by the bed partner, is due to an altered sound spectrum. Whether some acoustic aspects of snoring, such as changes in pitch, have predictive value for the presence of obstructive sleep apnea is at present not sufficiently substantiated.

Dirk Pevernagie and R.M. Aarts and Miche DeMeyer, The acoustics of snoring, Sleep Medicine Reviews, online Aug 8 2009, doi:10.1016/j.smrv.2009.06.002,Vol. 14, pp. 131-144, April 2010.

ENDOGENOUS MELATONIN RHYTHM BEFORE AND AFTER KIDNEY TRANSPLANTATION

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Introduction

The pineal hormone melatonin plays a pivotal role in the circadian sleep-wake rhythm. Recently, we have found that the nocturnal endogenous melatonin rise, which is associated with the onset of sleep propensity, is absent in many haemodialysis (HD) patients. Information on (changes of) the melatonin rhythm after kidney transplantation (kidney Tx) is limited.

Methods

In 7 HD patients, melatonin concentrations were measured. After kidney Tx, melatonin measurements were repeated in the same patients. Melatonin was measured in saliva samples. Samples were collected during 5 consecutive time-points at 9, 11 pm and 1, 7 and 9 am the next morning. Subjective sleep was measured with the Epworth Sleepiness Scale (ESS). The exposure to melatonin was estimated by calculating the area under the melatonin curve (AUC). The increase in melatonin production was calculated as the ratio of AUC after kidney Tx and AUC on HD.

Results

For general characteristics, see table 1. Before kidney Tx in all 7 patients a normal melatonin rhythm was absent. After kidney Tx in 4 out of the 7 subjects the nocturnal melatonin saliva concentrations reached normal levels of at least 4 pg/ml. Compared to their melatonin curves on HD, the melatonin concentrations after kidney Tx remained decreased in the 3 other patients. The subjective sleep score (ESS) of all patients after kidney Tx was significantly inversely correlated to the increase in melatonin production (R=-0.76; p=0.049).

Conclusion

Nocturnal endogenous melatonin concentrations normalized after kidney transplantation in 4 out of 7 patients. The mechanism whereby melatonin production is restored after kidney transplantation in some, but not all, patients needs to be evaluated in a larger study population. Such a study is currently planned. The significant correlation between subjective sleep score and melatonin rise warrants follow-up research on the use of exogenous melatonin and the management of sleep disturbances in patients after kidney transplantation who do not recover a healthy melatonin rhythm.

Table 1: General characteristics and results

Total no. (male)	7 (5)
Age (years ± sd)	51 ± 13
Time after Tx (months ± sd)	21 ± 13
ESS (points ± sd)	7 ± 5
AUCmelatonin after Tx : AUCmelatonin on HD ± sd	2.8 ± 3.3 *

* *R=-0.76; p=0.049*

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DAYTIME NAPPING AND EMOTIONAL AND DECLARATIVE MEMORY

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Introduction

Recent studies suggest that sleep benefits memory for emotional stimuli. However, it has also been suggested that sleep may attenuate emotional memories. This can only be understood when a dual role is assumed for sleep: the declarative memory part is consolidated, but associated emotions are decoupled. In previous studies on emotional memory REM sleep has been put forward as a candidate sleep stage for the consolidation of the declarative component (Wagner et al., 2001; Nishida et al., 2009). The effect of sleep on the emotional response towards a memory has not yet been investigated. In this study we examine the relation between different sleep stages and the retention of emotional and declarative components of memory.

Methods

The subjects (n=38) viewed emotionally neutral or distressing film fragments in the morning. During the ensuing 12-hour retention interval half of the subjects took a nap in the early afternoon and the other half in the late afternoon. During the nap EEG, EOG and EMG were recorded. The difference in circadian timing of the nap was assumed to result in more REM sleep and less slow wave sleep in the early nap compared to the late nap. This difference was confirmed with a trend for REM sleep ($p = 0.07$) and a significant decrease in SWS ($p < 0.01$). In the evening the memory of the subjects was cued with stills from the film. Emotional and physiological arousal state, as well as declarative memory, were assessed at the beginning and the end of the retention interval.

Results

The early nap group shows significantly less anger ($p < 0.05$) and trends towards less tension ($p = 0.06$) and depression ($p = 0.19$) in the evening than the late nap group. It can be concluded that the reinstatement of the emotional response in the evening is more strongly attenuated after the early nap. This might be due to the higher REM sleep percentage in the early nap. Opposite effects are found for declarative memory, which is superior after the early nap ($p<0.05$).

Conclusion

We find that the early nap has beneficial effects on the retention of declarative aspects of memory and, at the same time, attenuates emotional reactivation. These results show a dual function of sleep on memory, and suggest a prominent role of REM sleep in both functions. Previous studies on emotionally neutral memory suggest that sleep may particularly benefit retention when it follows shortly upon encoding. Therefore, an alternative explanation of our declarative memory findings regards the timing of the naps.

Presented at the 20th Congress of the European Sleep Research Society, September 2010, Lisbon

ADOPTING A USER-CENTERED DESIGN APPROACH TO DESIGN A PRODUCT THAT INFORMS PARENTS OF THEIR BABY'S SLEEP

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Introduction. Sleep rhythms of babies change a lot during the first year of life. Experts encourage parents to observe these rhythms and log them in sleep journals, particularly when problems arise. Some parents follow this advice. However, manually tracking sleep-wake rhythms is time consuming, the tracked rhythms not always reflect actual sleep time, and developments over time are difficult to inspect. To inform parents of their baby's sleep in a useful way, a product was designed using a user-centred design approach.

Methods. A user-centred design approach aims to produce useful and easy to use computer systems by early focus on users and tasks. Parent's needs for and initial reactions to a product that informs of their baby's sleep were studied by means of interviews and paper prototype evaluations. Then, a working prototype was developed that measured sleep rhythms through leg actigraphy and that automatically displayed information such as the amount of day and night sleep, sleep fragmentation, and sleep rhythm development. Six families with healthy babies (8-17 months) used the prototype for a week at home. Experts were interviewed.

Results. The user-centred approach allowed for a thorough understanding of parents' needs, context of use, reactions to the system, and how technology contributes to parents' security and curiosity needs surrounding their baby's sleep. Parents could imagine using the product 1) when they are not around, e.g., when the baby is in day care, 2) in case of sleep problems, 3) to get to know the rhythm, 4) to get insight in sleep development and share this information with caregivers, and 5) for fun. The product seemed particularly interesting to use with babies who sleep in day care, babies with sleep problems, and very young babies whose sleep rapidly develops. Expert's opinions varied; those that advice strict rhythms liked the product, and those that advice to rely on intuition did not. The relation between childrearing approach and product likeability should be examined in a larger population.

Conclusion. The user-centred approach appeared useful to design a product that informs parents on their baby's sleep. The evaluation in home settings allowed parents to really experience the product, and give useful feedback. Many parents and experts found an automatic sleep tracker useful for various reasons.

Presented at the 12th Annual international clinical symposium Kempenhaeghe.
To be presented at the ESRS conference in Lisbon, September 2010.

HOW TO USE ACTIGRAPHY: LIMITATIONS AND VALUE IN SLEEP MEDICINE

Al de Weerd

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Objective. Actigraphy (ACT) is a simple method to obtain data on sleep and wake. The presentation is meant to give an overview how to use ACT in sleep medicine.
Methods. The relevant literature on technical and clinical limitations will be discussed as well as the typical indications for ACT. The latter will be made clear using examples from clinical practice.
Results. As in each branch of medicine, the history given by the patient is the most important part of the work-up. By definition, details on sleep duration, wake periods, etc. are difficult to assess by the patient himself. Structured questionnaires and in particular ACT at home can help.
In comparison with the gold standard polysomnography (PSG), ACT gives no details on sleep architecture. Data easily obtainable from ACT as sleep onset latency, total sleep time, wake after sleep onset time, etc, often differ from those of a concurrent PSG. However, if ACT is done for at least 5 days and nights, the mean data on these sleep parameters are only slightly different from PSG. Due to its easy applicability, ACT is in particular useful in follow-up of patients with sleep disorders and in patients in whom PSG is not possible, for example in the mentally retarded. Furthermore, PSG followed by 1-2 weeks of ACT at home, provides background on sleep and wake in the normal situation of the patient, in addition to the details given by the PSG in the sleep laboratory. ACT has a major role in the analysis and follow up of young children with sleep disorders and in patients with abnormal function of the biological clock. The latter in combination with dedicated questionnaires and measurement of the Dim Light Melatonin Onset (DLMO).
Conclusion. ACT in itself and in addition to PSG is a mainstay in sleep medicine.

Lecture given at the ICCN, Kobe, October 2010

PERIODIC LIMB MOVEMENTS DURING SLEEP: ACTOR OR BYSTANDER?

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Objective. Periodic Limb Movements in Sleep (PLMS) have a prevalence up to 25% of the population, increasing with age. They often occur together with other disorders as Parkinsons disease, polyneuropathy, renal failure, rheumatoid arthritis, use of anti-depressants, etc. Eighty percent of patients with Restless Legs Syndrome (RLS) have PLMS. Still, a high percentage of patients with PLMS have no causative or related co-morbidity. In these cases the PLMS are often thought to be just concurrent with another disorder, for which polysomnography (PSG) was performed, for example insomnia. Some studies suggest that PLMS and the accompanying arousals from sleep may cause tiredness and excessive sleep during daytime, which still may introduce clinical significance for these events in the night . The presentation is meant to summarize the ongoing discussion if PLMS have clinical significance in cases with no RLS and if PLMS should be treated as a separate entity. Methods and results. The literature search will be discussed, but gives no clear answers. There is a tendency that PLMS should be considered as unspecific events during the night with no need for therapy except for patients with RLS. However, recent studies (Ferri et al., de Weerd et al.) suggest that subgroups of patients with insomnia can be delineated, in whom the PLMS have well defined characteristics (strict periodicity, highest prevalence in the first hours of sleep). Therapeutic options in these particular patients will be discussed. Conclusions. PLMS remain to be seen as unspecific phenomena during the night. The exceptions are concurrent RLS and possibly insomnia with specific characteristics of the accompanying PLMS.

Lecture given at the ICCN, Kobe, October 2010.

SLEEP IN VERY YOUNG CHILDREN WITH PRADER WILLI SYNDROME.
A STUDY BEFORE AND DURING GROWTH HORMONE SUBSTITUTION

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Objectives. Up to now sleep and respiration during sleep has been described in mixed populations of Prader Willi (PWS) patients, i.e. populations with large variations in age and treatment. Studies performed in children with PWS are limited to the older age categories. Our study aims at providing data on sleep and respiration during sleep in PWS children up to the age of 18 months and description of the effects of present day growth hormone (GH) therapy on the sleep of these young children.
Methods. All children with PWS born in The Netherlands are closely monitored beginning immediately after the diagnosis has been made, in nearly all cases a few days after birth. Part of the monitoring is full polysomnography (PSG) before the start of GH therapy. The registration is scheduled around the first birthday. A second PSG is done 7-10 months after the first GH dose and approximately one year after the first PSG .
Results. Twenty-two children participated (12 boys; age: median 11.5 months, IQR 10-14). All were healthy except for PWS and its direct consequences. Main first PSG data: sleep onset latency (SOL): median 20 min, IQR 11-40; REM latency (REM lat): 60, 40-111; REM duration (REM dur): 132, 120-173; REM%: 23, 20-28; deep sleep duration (SWS): 155, 137-163; SWS%: 26, 23-30; Stage 2 duration (St2dur): 247, 210-274; St2%: 42, 36-46; Sleep efficiency (SEI): 86, 80-88; Wake after sleeponset (WASO): 74, 51-116; Total Sleep Time (TST): 562, 550-604; Time in Bed (TIB): 681, 654-707; awakenings: 13, 10-15; AHI: 11, 8-13 of which 65, 55-83 % central apneas; 12 children with moderate or severe snoring; total leg movements: 52, 14-79; Periodic Limb Movements/hr (PLMI): 0, 0-0. At the second PSG SOL was similar; REMlat. was longer; REMdur. shorter and REM% lower; SWS shorter but SWS % similar; St2dur. similar and St2% higher; SEI similar; WASO shorter; TIB shorter, TST similar; awakenings similar; AHI and distribution of apneas similar. All differences significant at at least $p<0.05$
Conclusions. Sleep in one year old PWS patients, not yet on GH, is in all aspects similar to that of normal children except for a high AHI, in particular central apneas. The changes in sleep in the second year of life do not differ from those in control children (for the initial recording and the follow-up: all comparisons to our database of sleep in normal children and their sleep development: non significant). In these young PWS patients the severity of apneas is not influenced by GH.

Free communication ESRS, Lisbon, September 2010

EPILEPSY IN CHILDREN…WHAT ABOUT THEIR SLEEP?

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Purpose: Sleep disturbances are common in adults with epilepsy. For children sleep is often abnormal too, but exact data are not available. The aim of the study is to provide more insight in the interaction epilepsy and sleep during childhood.
Method: The parents of 4-10 y/o children with partial epilepsies (N=120) were asked to participate. The children visited normal school or one level below. They used no more than two anti-epileptic drugs (AEDs). These requirements precluded participation of severely handicapped children. All were patients in our specialized epilepsy center. The WHO epilepsy questionnaire, Medical Outcome Score-Sleep (MOS), Sleep Disturbance Scale for Children (Bruni list) and a severity scale for epilepsy in children (HSSC) were used. Every child brought in a healthy control of similar age whose parents filled in the same questionnaires on the aspects of sleep.
Results: Patients and controls differed substantially ($p<0.05$-$p<.001$). Initiating and maintaining sleep was abnormal in 40% of the patients and in 15% of controls, For parasomnia's and sleepiness during the day these figures were 32-21% and 38-18%, respectively. There were no differences for the respiratory parameters. The total score on the Bruni list was abnormal in 35% of the patients and in 17% of controls with similar data for the MOS.
Conclusion: In children with epilepsy about twice as much sleep disturbances were encountered when compared to controls, in particular regarding initiating and maintaining sleep, parasomnia and sleepiness during the day.

Free communication during IPSA, Rome, December 2010

CHARACTERISTICS OF NEAR SKIN TEMPERATURES IN THE BED

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Introduction. Recent studies on the interaction of sleep and body temperature show that skin temperature affects sleep and that minor changes in skin temperature can have a major impact on sleep architecture and sleep onset. Skin temperature is affected by the thermophysiological state of the body, which subsequently affects the temperature in the bed. This sleep microclimate is in turn determined by factors like the isolative properties of the duvet. Not much is known on the bed climate in a regular bedroom setting at home. We explored the characteristics of near skin temperatures in the bed.

Methods. 20 subjects without sleep complaints participated in the study; mean age was 32 years, 5 females. Subjects were monitored for 2 nights in their home environment and they were advised to go to bed at habitual bed time. Sleep was monitored using a frontal EEG-EOG based sleep monitor (Zeo Inc, USA), wrist actigraphy (Philips Respironics, USA) and subjective reports. Temperature in bed was monitored using 16 Ibuttons (DS1923; Maxim/Dallas Semiconductor Corp, USA) equally distributed on the inside of a duvet cover. For preliminary analyses we selected the data of the 4 central Ibuttons (near trunk of the body). Temperature in the bed just before entering the bed, the steady temperature after the initial increase and overnight range, maximum and average in temperatures were determined. The change of the temperature in bed was quantified by time to warm up the bed, increase in bed temperature and speed of bed warming.

Results. The temperatures under the blanket near the trunk ranged from 10.6 °C to 37.1 °C, with an overnight average of 32.7 °C. The average maximum overnight bed temperature was 34.7°C. The coolest bed temperature was always recorded at the moment of entering the bed (on average 19.1 °C). It took on average 69 minutes to reach a stable temperature of 33.2 °C. Within these 69 minutes the temperature increased on average 14.1 °C and this equals an average of a quarter degree Celsius per minute. All temperature measures show large individual differences. Correlation statistics with objective and subjective sleep measure are in preparation.

Conclusion. Bed temperatures measured in the home setting show large individual differences. These differences probably reflect both thermophysiological differences and environmental differences like room temperature and isolative properties of the duvet.

Will be presented as a poster at the 20th Congress of the European Sleep Research Society, Lisbon, Portugal (2010).

DIFFERENCES IN HABITUAL BED TEMPERATURES OF MEN AND WOMEN

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Introduction. From interviews on temperature perception during sleep initiation it is known that many males are complaining of a rather too warm feeling when attempting to fall asleep whereas women more often complain of having cold feet. To our knowledge there is not much known on gender difference in skin temperatures or bed temperature, although it is known that the nocturnal drop in core body temperature is relatively blunted in women as compared to men. We explored the gender differences in the characteristics of near skin temperatures in the bed to see if the subjective reports mentioned earlier on the sensation of the temperature in bed is reflected as well in objective temperature data.

Methods. We explored a sample of age matched subjects, 5 males and 5 females, that was selected out of participants without sleep complaints of a broader study on temperature in bed. Subjects were monitored for 2 nights in their home environment and they were advised to go to bed at habitual bed time. Temperature in bed was monitored using 16 Ibuttons (DS1923; Maxim/Dallas Semiconductor Corp, USA) equally distributed on the inside of a duvet cover. For preliminary analyses we selected the data of the 4 central Ibuttons (near trunk of the body). Temperature in the bed just before entering the bed, the stable temperature following the initial increase and overnight range, maximum and average in temperatures were determined. The change of the temperature in bed was quantified by time to warm up the bed, increase in bed temperature and speed of bed warming.

Results. Within this small sample we observed a difference in the average overnight temperature of the bed. We observed an overnight average of 32.1 °C in the sample of males as compared to a 33.3 °C in the sample of females ($p<0.05$). No differences were observed in the initial bed temperature, time to warm up the bed, increase in bed temperature or the rate of the warming of the bed.

Conclusion. We did not find objective proof for the subjectively reported difference in temperature perception during sleep initiation. Analyses of the bed temperatures near the foot area might reveal gender differences and these data are in progress.

Will be presented as a poster at the 20th Congress of the European Sleep Research Society, Lisbon, Portugal (2010).

IS THE TEMPERATURE IN YOUR BED RELATED TO SLEEP ONSET?

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Introduction. Sleep onset latency is related to the rise is skin temperature and the drop in core body temperature. This rise in skin temperature might be hampered by entering a relatively cool bed that might result in a vasoconstriction of the skin and hence drop in skin temperature. We measured the gradual increase of the near skin temperature in the bed and explored if it was correlated to the sleep onset latency.

Methods. 17 subjects without sleep complaints participated in the study, mean age was 33 years. Subjects were monitored for 2 nights in their home environment and they were advised to go to bed at habitual bed time. Sleep was monitored using a frontal EEG-EMG-EOG based sleep monitor (Zeo Inc, USA). Sleep onset was based on the Time to Z output of the ZEO system, reflecting time to fall asleep. Temperature in bed was monitored using 16 Ibuttons (DS1923; Maxim/Dallas Semiconductor Corp, USA) equally distributed on the inside of a duvet cover. For preliminary analyses we selected the data of the 4 central Ibuttons (near trunk of the body) and the 2 lower central Ibuttons (near the feet). Temperature in the bed just before entering the bed and the steady temperature after the initial rise were determined. The change of the temperature in bed was quantified by time to warm up the bed, increase in bed temperature and speed of bed warming.

Results. Time to fall asleep was negatively affected by the time to warm up the bed. The faster the increase of the bed temperature near the feet, the shorter it takes to fall asleep ($p =< .05$). Changes in temperature near the trunk of the body did not significantly affect sleep onset. The effect of the initial temperature in the bed near the trunk (warmer bed associated with shorter sleep latencies) only reached trend level. No other effects could be observed.

Conclusion. The rate of change in the bed temperature at the feet area is caused by the temperature of the feet, warming up that particular bed area. Our observation is in line with previous results showing that faster increases of foot temperature after lights-off seemed to be involved in shortening sleep onset latency, suggesting a role for the rate of change rather than the level of distal temperature.

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CORRECTION FOR MODEL SELECTION BIAS USING A MODIFIED MODEL AVERAGING APPROACH FOR SUPERVISED LEARNING METHODS APPLIED TO EEG EXPERIMENTS

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This paper proposes a modified model averaging approach for linear discriminant analysis. This approach is used in combination with a doubly hierarchical supervised learning analysis and applied to preclinical pharmaco-electroencephalographical data for classification of psychotropic drugs (including sleep medication). Classification of a test dataset was highly improved with this method.

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CIRCADIAN REGULATION OF SLEEP AND THE SLEEP EEG UNDER CONSTANT SLEEP PRESSURE IN THE RAT

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Introduction. Sleep is regulated by homeostatic and circadian processes. Slow wave activity (SWA; 1-4 Hz) in the NREM sleep electroencephalogram (EEG) reflects sleep homeostasis. Activity of faster EEG frequencies (10-25 Hz) is thought to be under influence of circadian factors. The relative contribution of both processes to the distribution of sleep and wakefulness and EEG activity in rodents remains uncertain.

Methods. Continuous EEG recording in rats in constant dark conditions (DD) were performed and a sleep deprivation protocol consisting of 2 h sleep deprivation followed by 2 h of rest (2h/2h) was applied for 48 h to obtain a constant sleep pressure.

Results. Under the 2h/2h protocol, the circadian modulation of waking, NREM and REM sleep was markedly reduced compared to the baseline, affecting the frequency of vigilance state episodes and the duration of REM sleep and waking episodes. In contrast, NREM sleep episode duration still showed a daily modulation. Consecutive 2h values of SWA in NREM sleep were stabile during the 2h\2h protocol, while NREM sleep EEG activity within the higher frequencies (7-25 Hz) still demonstrated strong circadian modulation, which did not differ from baseline.

Conclusions. In rats, the daily modulation of REM sleep is less pronounced compared to NREM sleep and waking. In contrast to SWA, activity in higher frequencies (7-25 Hz) in the NREM sleep EEG have an endogenous circadian origin and are not influenced by sleep homeostatic mechanisms.

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INTERRELATIONS OF SLOW AND HIGH FREQUENCY ACTIVITY IN THE NREM SLEEP EEG IN THE RAT

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Introduction. Under constant sleep pressure, which can be achieved by repeated short-sleep deprivation, the marker of the sleep homeostat - slow wave activity (SWA, 1-4 Hz) in the NREM sleep electroencephalogram (EEG) - tends to stabilize its circadian modulation, while higher frequencies (7-25 Hz) preserve their daily rhythm, therefore demonstrating a dependency on the endogenous circadian clock (Yasenkov&Deboer, 2010). To further investigate the interrelations between different EEG frequencies in NREM sleep under these conditions, we applied a correlation analysis for the power density data in 1 Hz bin.

Methods. EEG and electromyogram recordings were simultaneously performed in freely moving rats (n=8) adapted to constant darkness. A 24h baseline day (BL) was recorded, followed by 48 hours of the repeated short-sleep deprivation protocol (2h/2h), which consisted of 2h periods of sleep deprivation, alternating with 2h periods of rest. Vigilance states were determined and spectral analysis of the NREM sleep EEG for the frequency range 0.5-25 Hz was performed in 1 Hz bin. Subsequently, the obtained power density data over 4-h intervals of the last 24h in 2h/2h and BL were assessed for bivariate correlation analysis (Pearson's correlation coefficient, r), then r-values were Fisher-Z transformed, averaged across all animals and retransformed.

Results. Spectral analysis of the NREM sleep EEG during the BL day showed a clear circadian pattern for each 1 Hz bin within SWA (rANOVA, factor interval, $p<0.01$). Higher frequencies (7-25 Hz) also demonstrated a circadian modulation (rANOVA, $p<0.01$), but in an opposite direction, gradually increasing the power during the rest phase and declining during the active phase. The 2h/2h protocol stabilized the level of EEG power density within SWA (rANOVA, factor interval, $p>0.05$), and reduced its circadian amplitude, but did not affect the daily changes of frequencies above 7 Hz compared to BL (rANOVA, factor interval, $p<0.01$; factor day, $p>0.05$; for all bins).A correlation matrix for the NREM sleep EEG power density during BL revealed significant positive relationships for 1 Hz bins within three frequency bands: SWA (1-4 Hz), 9-14 Hz and 15-25 Hz (all 1 Hz bin comparisons $p<0.001$). In addition, a tendency for a negative relationship between SWA and higher frequencies (8-25 Hz) was observed. Under the 2h/2h protocol correlation levels remained intact within SWA, but increased in the higher frequency range where significant values were obtained within one extensive frequency band (8-25 Hz; $p<0.001$).

Conclusions. In NREM sleep during BL three separate correlation clusters were found, which may represent slow-waves (1-4 Hz), spindle frequency activity in rats (7-14 Hz), and high frequencies (15-25 Hz) and which responded differently to repeated short-sleep deprivation. Under constant sleep pressure, the absence of changes in correlation level within SWA confirms its homeostatic component involved in sleep regulation, while a pronounced increase of correlation coefficients within 8-25 Hz depicts an endogenous circadian influence on higher frequencies activity in EEG.

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