Circadian Rhythmicity and Epilepsy:
the Significance of Chronobiological Time

Wytske Æ. Hofstra

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Cover: “Head of a Woman” (1937-38). Estate of Pablo Picasso.
ISBN: 9789086595372
Printed by: Ipskamp Drukkers

The studies in this thesis were designed and carried out at the Epilepsy and Sleep Centre SEIN Zwolle, The Netherlands, the Rudolf Magnus Institute of Neuroscience, University Medical Centre, Utrecht, The Netherlands and the Medical School Twente, Medisch Spectrum Twente Hospital, Enschede, The Netherlands.

This research was financially supported by the ‘Christelijke Vereniging voor de Verpleging van Lijders aan Epilepsie’.

Financial support for the printing of this thesis was kindly provided by: Stiching Epilepsy Instellingen Nederland; Stichting Het Remmert Adriaan Laan-Fonds, Amsterdam; the VU University, Amsterdam; Stichting Neurologisch Onderzoek Twente, Enschede; UCB Pharma; the Nederlandse Vereniging voor Slaap Waak Onderzoek; Glaxo SmithKline BV and the Hoytema Stichting.

VRIJE UNIVERSITEIT

Circadian Rhythmicity and Epilepsy: the Significance of Chronobiological Time

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op vrijdag 4 maart 2011 om 11.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Wytske Ætske Hofstra

geboren te Bedum

promotor: prof.dr. C.J. Stam
copromotor: dr. A.W. de Weerd

List of abbreviations

AASM American Academy of Sleep Medicine
AED anti-epileptic drugs
BP blood pressure
CBT core body temperature
CBZ carbamazepine
CPS complex partial seizures
CTI Circadian Type Inventory
CTQ Circadian Type Questionnaire
DLMO dim light melatonin onset
DTS Diurnal Type Scale
ECG Electrocardiography
ECoG Electrocorticography
EEG Electroencephalography
EMG Electromyography
EOG Electrooculography
F frontal
FLE frontal lobe epilepsy
GTCS generalized tonic clonic seizures
HRV heart rate variability
I-EEG intracranial electroencephalography
IED interictal epileptic discharge
IEM intracranial electrocorticography monitoring
JME juvenile myoclonic epilepsy
LTLE lesional temporal lobe epilepsy
Lx Lux
MCTQ Munich Chronotype Questionnaire
MEQ Morningness Eveningness Questionnaire
MLT melatonin
MSF mid sleep on free days
MSFsc corrected mid sleep on free days
MTLE mesial temporal lobe epilepsy
NREM non-rapid eye movement
NTLE neocortical temporal lobe epilepsy
O occipital
P parietal
PSG polysomnography
REM rapid eye movement
SCN suprachiasmatic nuclei
S-EEG superficial electroencephalography
SUDEP sudden unexpected death in epilepsy
TLE temporal lobe epilepsy
TSTF total sleep duration on free days
VP vasopressin
XTLE extratemporal lobe epilepsy

Contents

Chapter One

General introduction, aim and outline of this thesis

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General introduction

Epilepsy is a common neurological disorder, in which the normal pattern of neuronal activity can become disturbed. This leads to seizures with strange sensations, emotions, muscle spasms and/ or loss of consciousness. According to the World Health Organization it is the most common serious brain disorder worldwide, with 40 to 50 million patients in the world today and an estimated two million new cases each year. In the Netherlands an estimated 121000 people suffer from epilepsy, compared to for example 16000 patients with Multiple Sclerosis and 26000 patients with Parkinson's disease (numbers 2008 (Gommer and Poos, 2010a, b and c)).

Epilepsy has been focus of intensive research over many decades now, yet many pathophysiological and physiological questions remain. Furthermore, despite the development of various anti-epileptic drugs and the introduction of epilepsy surgery, vagal nerve stimulation and deep brain stimulation, many epilepsy patients continue to have seizures. Therefore, more research is needed in order to further understand epilepsy and improve seizure control.

There are many factors that influence seizure occurrence, one of which is sleep. It is well known that sleep deprivation can lead to an increase in seizure frequency. The relation of sleep and seizures has been studied thoroughly. Despite all knowledge on sleep and epilepsy, knowledge on circadian rhythmicity and epilepsy is poor.

Circadian rhythms are endogenously mediated 24 hour cycles of physiological and psychological processes, including sleep-wake cycle, core body temperature, blood pressure, task performance and hormone production (Hastings et al., 2007). The influence of circadian rhythms is suspected in several diseases, including cancer. Knowledge about the influence of the circadian rhythm on human epilepsy and seizures and vice versa is, however, relatively scarce. If such an interaction exists, it may be of value for better knowledge of pathophysiology and for timing of diagnostic procedures and therapy. It appears that human seizure occurrence may have 24-h rhythmicity, depending on the origin, i.e. the brain lobe the seizure begins (Hofstra et al., 2009a; Hofstra et al., 2009b; Pavlova et al., 2004; Quigg et al., 1998b; Quigg and Straume, 2000). These findings are supported by animal studies. Rats placed in constant darkness showed spontaneous limbic seizures occurring in an endogenously mediated circadian pattern (Quigg et al., 2000). More studies are available on the influence of epilepsy on circadian rhythms. Significant differences in timing of daily activities, sleeping and wakefulness (so-called chronotype) between patients with different epilepsy syndromes have been found and numerous studies have described influences of epilepsy and seizures on sleep. In contrast, knowledge on (core) body temperature and clock genes in patients is minimal. Reduced heart rate variability and changed hormone levels, which are under the influence of the biological clock, have been observed in people with epilepsy. In short, large gaps in the knowledge about the interaction of circadian rhythm and human epilepsy still remain.

Aim of this thesis

In this research, we aimed to elucidate the relationship between circadian rhythmicity and epilepsy. If this interaction between circadian rhythm and epilepsy exists, it may be of value for better understanding of the pathophysiology of epilepsy. Furthermore, it may especially be important for the timing of diagnostic procedures, such as electroencephalography (EEG) and therapeutic options. We specifically focused on (human) seizure rhythmicity, both 24 hour and circadian and also on chronotypes and the possibilities of chronotherapy in epilepsy patients.

Questions to be answered in this thesis are:

- Is there rhythmicity in human seizures? Are these rhythms different for the various types of seizures? Are these rhythms different for the origin of seizures?
- If there is rhythmicity in human seizures, is there proof that this might be a circadian rhythm?
- Is chronotype, also a circadianly mediated rhythm, affected by epilepsy? That is, are chronotypes differently distributed in epilepsy patients compared to controls? And more specific, do patients with different epilepsy syndromes have a different distribution of chronotypes?
- Do epilepsy patients with different chronotypes take their medication on the same times? Or do, for example, evening types take their anti-epileptic drugs on later times than morning types do?

Outline of this thesis

In Chapter 2 and 3 further introduction is given on the current knowledge of the interaction between circadian rhythmicity and epilepsy and how circadian rhythmicity is measured in humans. Chapter 4 and 5 focus on rhythmicity in seizures on the 24 hour day as measured by surface EEG and intracranial EEG: what seizure rhythmicity is found and is this different for different types and origins of seizures. In Chapter 6 the distribution of chronotypes and certain sleep parameters in epilepsy patients is studied. In this chapter it is investigated whether epilepsy patients have different chronotypes and sleep parameters compared to controls without epilepsy. Patients with different epilepsy syndromes are also compared. In chapter 7 the timing of taking anti-epileptic drugs in the various chronotypes is described. Do these times differ between patients with different chronotypes or do patients take their medication according to the advice of the physician? Finally, chapter 8 focuses on the timing of seizures when measured in relation to the circadian phase of the individual patient. Is there a link with circadian rhythmicity or is seizure rhythmicity in humans a diurnal instead of circadian rhythm?

Finally, a summary of the thesis is presented in English and Dutch and further perspectives are discussed.

Chapter Two

The circadian rhythm and its interaction with human epilepsy: A review of literature

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Sleep Medicine Reviews (2009) 413-420

Abstract

Knowledge on the interaction between circadian rhythm and human epilepsy is relatively poor, although if it exists, this interaction may be of value for better knowledge of pathophysiology and for timing of diagnostic procedures and therapy. It appears that human seizure occurrence may have 24-hour rhythmicity, depending on the origin. These findings are endorsed by animal studies. Rats placed in constant darkness showed spontaneous limbic seizures occurring in an endogenously mediated circadian pattern. More studies are available on the influence of epilepsy on circadian rhythms. Significant differences in chronotypes between patients with different epilepsy syndromes have been found and numerous studies have described influences of epilepsy and seizures on sleep. In contrast, knowledge on (core) body temperature and clock genes in patients is minimal. Reduced heart rate variability and changed hormone levels, which are under the influence of the biological clock, have been observed in people with epilepsy. In short, large gaps in the knowledge about the interaction of circadian rhythm and human epilepsy still remain. Proposals for studies in this borderline area between the biological clock and epilepsy will be discussed.

Background

It is well known that sleep can influence epilepsy and epileptic seizures. Knowledge about the influence of the circadian rhythm on human epilepsy and seizures and vice versa is, however, relatively scarce. If this interaction between circadian rhythm and epilepsy exists, it may be of value for better understanding of the pathophysiology of epilepsy. Furthermore, it may especially be important for the timing of diagnostic procedures and therapy.

Outline

The aim of this systematic review is to present an overview of recent knowledge of circadian rhythms in human epilepsy. Where this contributes to insight into this interaction in humans, a short introduction on knowledge from animal studies is included. A literature search was performed in PubMed. Per topic keywords have been used to search in this database, with limits on the publication date (from 1-1-1980 onwards) and language (English). For example, when considering core body temperature in epilepsy the search was performed with the words “core body temperature epilep*” in different combinations. Cross-references have also been included.

Circadian rhythm and its measurement in humans are briefly discussed and the current state of knowledge of circadian rhythm in human epilepsy is described. This includes 24 hour (24-h) rhythmicity in seizures and interictal discharges as well as current knowledge on clock genes in epilepsy. Studies on the influence of epilepsy and seizures on circadian rhythms are reviewed and a brief overview of the distribution of chronotypes in people with epilepsy and the influence of epilepsy and seizures on sleep and the sleep-wake cycle is discussed. Data on core body temperature in epilepsy is reviewed and changes in cardiovascular parameters in patients with epilepsy are discussed. Temporal rhythms of the hormones melatonin, cortisol, prolactin and growth hormone in relationship to epilepsy and seizures are assessed. Finally, options for further research will be given.

Circadian rhythm

Circadian rhythms are endogenously mediated ~24-h cycles of physiological and psychological processes, including the sleep-wake cycle, core body temperature, blood pressure, task performance and hormone production. These circadian rhythms in mammals are generated and maintained by a biological clock in which the master circadian pacemaker is formed by the cells of suprachiasmatic nuclei (SCN). As well as the master pacemaker in the SCN, there is convincing evidence for the existence of peripheral circadian oscillators in the human body. More or less independent peripheral oscillators are found in several organs, including the liver, skeletal muscle and testis; all are under the influence of the SCN (Lamont et al., 2007). To synchronize the circadian system to the 24-h day, the SCN need to adjust daily. This is termed entrainment and this is accomplished by external cues, so-called Zeitgebers (“time givers” in German).

Several genes have been discovered that are at least partly responsible for this characteristic activity of the individual SCN and the interindividual differences. The activity depends on the expression of auto regulatory translation-transcription feedback loops of genes including the *Period* genes (*Per1, Per2, Per3*), the *Clock* gene and two *Cryptochrome* genes (*Cry1, Cry2*). It has been demonstrated in several animal studies that deletion or mutation of these genes leads to rhythms with abnormal periods or even arrhythmic

phenotypes when tested under constant conditions. Moreover, dysfunction of these clock genes might be important in the development of various diseases, including cancer (Lamont et al., 2007).

How to measure the circadian rhythm in humans

In theory, all circadian output rhythms driven by the biological clock that can be measured can be used to assess the circadian rhythm. In research and clinical practice, however, melatonin production, core body temperature, cortisol production and questionnaires are most frequently used.

Measurement of the whole 24-h rhythm of melatonin is considered to be the most robust phase marker. This is time-consuming and inconvenient and therefore, the time at which melatonin production starts rising in the evening is used more often as indication of the circadian phase. Dim lit surroundings are important as light intensities as low as ~100-180 lux (room light) can suppress production of melatonin. For this reason, the procedure is termed the dim light melatonin onset (DLMO) measurement (figure 1A) (Lewy et al., 1999).

The circadian rhythm of the core body temperature (CBT) is characterized by a nocturnal decline, with a minimum temperature at approximately 5 a.m.. The temperature rises during the day and reaches its maximum at around 5 p.m. (figure 1B). The circadian rhythm of a subject can be measured relatively easily through collection of continuous CBT data.

Cortisol is a corticosteroid hormone that is secreted in a highly rhythmic fashion, with declining levels throughout the day, a nocturnal period of quiescence and a sharp rise in the second half of the night toward a morning maximum (figure 1C). The nadir is reached within approximately 2 hours after sleep onset.

It is important to realize that many factors can influence these three measurements, thereby masking the true endogenous signal. Such masking factors affecting the DLMO include posture, exercise, sleep, caffeine and certain drugs. Postural changes, physical activity, meals, ambient temperature, sound, humidity and bright light can influence CBT and physical and physiological stress, light, age, sleep and high protein meals can influence the cortisol level (Hofstra and de Weerd, 2008). It is crucial to minimize these disturbing factors in order to measure true circadian rhythms. The CBT in particular is under the influence of many masking factors that are difficult to control in ‘real life’. This makes the use of constant routine procedures indispensable for including CBT as a marker of the circadian rhythm. As a consequence, this method is not often used in clinical research and the other methods are preferred.

Questionnaires have been developed to study individual characteristics concerning timing of daily activities and sleep (so-called chronotype). The Morningness Eveningness Questionnaire (MEQ) by Horne and Ostberg (1976) differentiates morning and evening type individuals and this reflects part of the circadian rhythm (Horne and Ostberg, 1976). A very large recent review concluded that the MEQ appears to be a fair predictor of the endogenous circadian phase or period (Sack et al., 2007). Together with the MEQ, the Munich Chronotype Questionnaire (MCTQ) by Roenneberg (2003) is useful in determining the chronotype in a general population (Roenneberg et al., 2003), in particular because the MCTQ takes differences between working and free days into account.

Circadian rhythms in epilepsy

Twenty-four hour rhythmicity in seizure occurrence

In animal studies clear diurnal patterns of seizures in various epilepsy models have been observed. For example, in studies of rodents with limbic epilepsy it was observed that seizure latency was shorter and more spontaneous seizures occurred during exposure to light than during darkness (Bertram and Cornett, 1994; Cavalheiro et al., 1991; Gorter et al., 2001; Hellier and Dudek, 1999; Quigg et al., 1998b; Quigg et al., 2000). In an interesting study, epileptic rats were monitored with constant EEG recording. The rats were entrained to a 12 hour-12 hour light-dark cycle and then exposed to constant darkness, in order to get a free running circadian rhythm. During the light-dark exposure, spontaneous seizures originating in the limbic system (amongst others comprising the hippocampus and amygdala) occurred in statistically non-uniform patterns with nearly twice as many seizures occurring during the light period. During constant darkness, seizures continued to occur in the same pattern observed during the light-dark study, after correction for the circadian rhythm of the body temperature. These findings clarify that spontaneous limbic seizures in rats occur in a true endogenously mediated circadian pattern (Quigg et al., 2000).

Over a century ago, Gowers classified seizure occurrence as diurnal, nocturnal and diffuse (Gowers, 1885). Later studies confirmed and extended his findings. Well known examples are the strong association with the night in seizures in some frontal lobe syndromes (e.g. autosomal dominant nocturnal frontal lobe epilepsy) and myoclonic seizures in juvenile myoclonic epilepsy that occur predominantly after awakening in the morning (Panayiotopoulos et al., 1994; Scheffer et al., 1995).

Few studies have included more detailed temporal distribution of seizures over the 24-h day (table 1). One case report based on a seizure diary maintained for five years of a subject with two epileptic foci showed that both temporal and parietal seizures occurred independently from each other in non-random, daily patterns (Quigg and Straume, 2000). Peak incidences were found at 1210h in temporal seizures and at 0250h in parietal seizures. Three retrospective studies have used the more reliable continuous EEG monitoring, to evaluate seizure occurrence (Hofstra et al., 2009a; Pavlova et al., 2004; Quigg et al., 1998b). In one study, patterns of seizures in 64 patients with mesial temporal lobe epilepsy (mesial TLE, MTLE, i.e. mesio basal temporal structures), 26 with extraTLE (XTLE) and 8 with lesional TLE (LTLE) were studied. The results were compared to the occurrence of seizures in a rat model of limbic epilepsy (Quigg et al., 1998b). It was found that seizures in LTLE and XTLE occur randomly. In patients with MTLE seizures occurred in a daily cosinor distribution with a peak incidence at approximately 1500h. This was comparable to the distribution in time of seizures observed in the rats. In another study, Pavlova et al. described 26 patients (90 seizures) and found a significant peak in seizure occurrence between 1500 and 1900h in TLE patients and a peak between 1900 and 2300h in XTLE patients (Pavlova et al., 2004). In our own tertiary epilepsy centre, we have evaluated the temporal distribution of 808 clinical seizures in 100 adults and 76 children with partial epilepsies (Hofstra et al., 2009a).

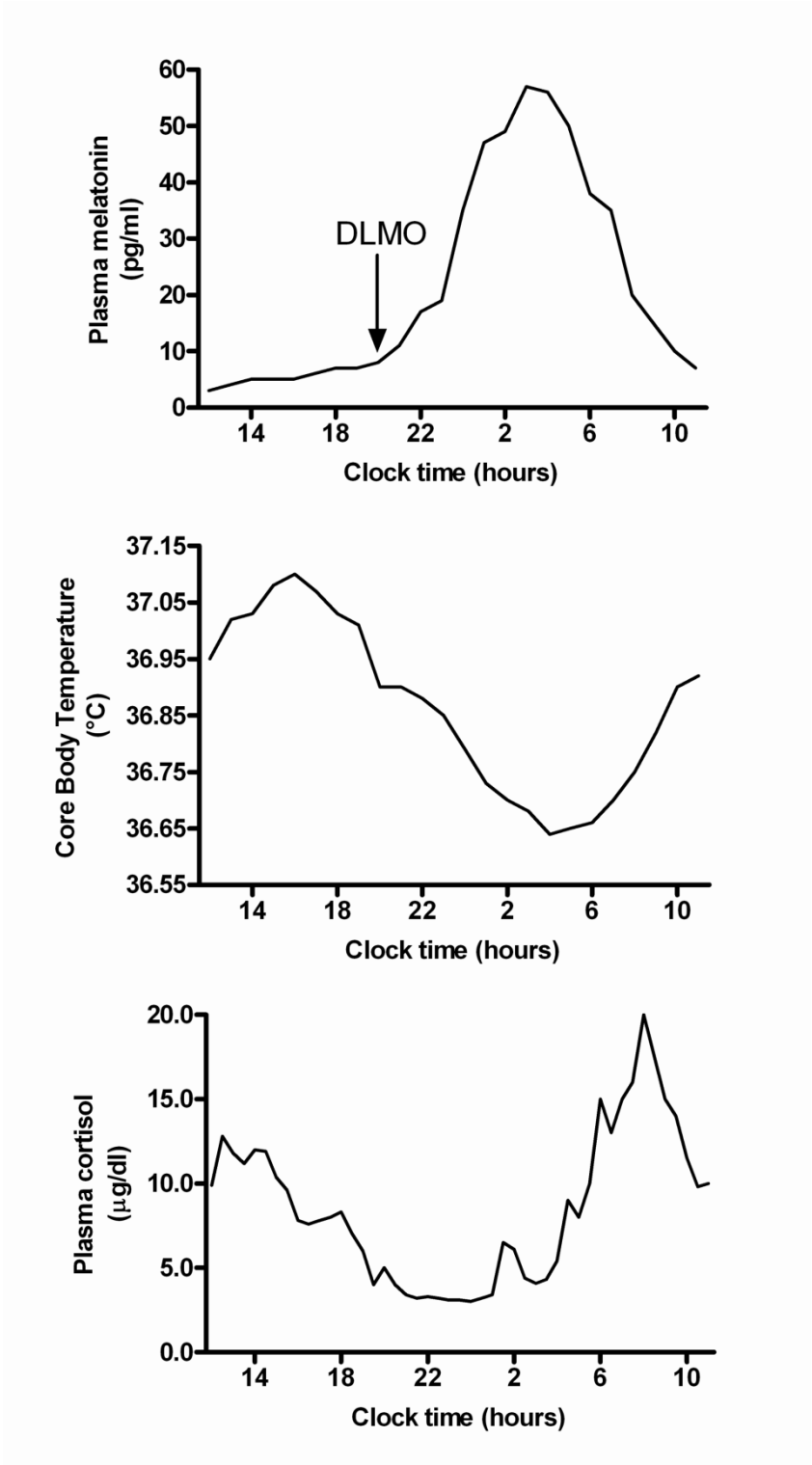

Figure 1. Human circadian rhythms of the plasma melatonin level (A), core body temperature (B) and plasma cortisol level (C). Dim light melatonin onset (DLMO) is indicated in (A). Data adapted from (Hofstra and de Weerd, 2008).

Significantly more seizures were observed from 1100 to 1700h and from 2300 to 0500h fewer seizures were seen. Daytime peak incidences were observed for all types of seizures and more differentiated for complex partial seizures, seizures of extratemporal origin (in children) and seizures of temporal origin (in adults). The total number of seizures, complex partial seizures and tonic seizures (in children) were low in the period 2300 to 0500h. Also, significantly fewer seizures of temporal and extratemporal origin (in children) were observed in this period. For more detailed distribution of complex partial seizures, see figure 2. Two studies have been performed in which intracranial EEG monitoring (considered the gold standard) was used. In one paper 669 seizures of 131 adult patients were analysed (Durazzo et al., 2008). Non-uniform distributions were observed in seizures from the parietal, occipital, mesial temporal and neocortical temporal lobes. Occipital seizures peaked between 1600 and 1900h; parietal and frontal lobe seizures peaked between 0400 and 0700h. Two peaks were found in the occurrence of seizures from the mesial temporal lobe (1600-1900h and 0700-1000h). Seizures from the neocortical temporal lobe also peaked between 1600 and 1900h. In another study, we have analysed 450 spontaneous seizures in 33 patients with long term intracranial EEG and video monitoring. Seizures showed an uneven distribution over the day, depending on lobe of origin: temporal lobe seizures occurred preferentially between 1100 and 1700h, frontal seizures between 2300 and 0500h and parietal seizures between 1700 and 2300h (Hofstra et al., 2009b).

Twenty-four hour rhythmicity in the occurrence of interictal EEG activity

Epileptiform phenomena on the EEG in seizure free periods (interictal epileptic discharges, IEDs) have been studied extensively in humans. Several groups have found that the number of IEDs increases significantly during sleep, in parallel with ultradian 100-min cycles of REM/ NREM (Shouse et al., 1996). During NREM sleep (especially light NREM stages), focal and generalized IEDs are common. Although attenuating during REM sleep, focal IEDs persist, but generalized IEDs are infrequent in this sleep stage. None of these studies, however, has regarded the pure influence of circadian rhythm itself, as the results may be masked by the sleep-wake cycle. Thus these studies give insight in the influence of sleep on IEDs, but not into the contribution of the endogenous 24-h rhythm.

Genes

It has been shown that the loss of circadian PAR bZIP transcription factors in mice may result in severe epilepsy (Gachon et al., 2004). These factors are transcriptionally controlled by the circadian molecular oscillator and are thought to be under the influence of the circadian clock. Although this is a very interesting finding for insight into the interaction between the circadian rhythm and epilepsy, the precise relevance of this study for human epilepsies remains to be elucidated.

No studies have been published regarding so-called clock genes in patients with epilepsy.

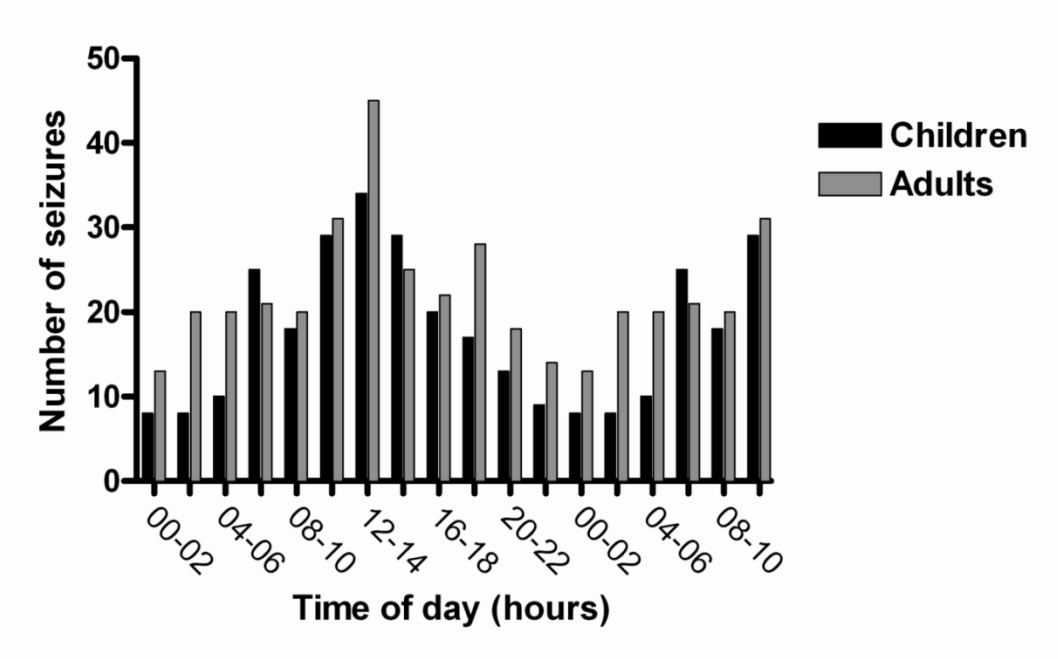

Figure 2. Bar histogram showing temporal distribution of complex partial seizures in children and adults (numbers of seizures are n=215 and n=265 respectively). Each bar represents numbers of seizures per two hours. Data of 36 hours is given in order to show the 24 hour cosinor rhythmicity. Data adapted from (Hofstra et al., 2009a).

Table 1. Studies on 24 hour rhythmicity in human seizure occurrence

Reference	Patients *n*=	Methods	Seizures *n*=	Results	Detailed temporal distribution
(Quigg et al., 1998b)	96	S- EEG	MTLE 774; XTLE 465; LTLE 48	Random and Non-random	Random seizure patterns in LTLE and XTLE. Distinct non-random seizure pattern in MTLE (peak 1500h)
(Quigg and Straume, 2000)	1¥	Seizure diary	TLE 694; P 315	Non-random	Non-random daily patterns in seizures from two epileptic foci. Peak of temporal seizures at 1210h and of parietal seizures at 0250h
(Pavlova et al., 2004)	26	S- EEG	TLE 41; XTLE 49	Non-random	Significant peak from 1500-1900h in TLE; peak from 1900-2300h XTLE
(Durazzo et al., 2008)	131	I- EEG	MTLE 217; NTLE 160; P 77; F 132; O 83	Non-random	Non-uniform seizure distributions in P; O; MTLE; NTLE. Peak in O 1600-1900h, peak in P and F 0400-0700h. MTLE 1600-1900h and 0700-1000h; peak in NTLE 1600-1900h
(Hofstra et al., 2009a)	176	S- EEG	TLE 315; XTLE 493	Non-random	Daytime peaks (1100- 1700h) in all types of seizures; CPS; XTLE (children); TLE (adults); Night time lows (2300-0500h) in all types of seizures; CPS; tonic seizures (children); TLE; XTLE (children)
(Hofstra et al., 2009b)	33	I-EEG	MTLE 85; NTLE 72; P 99; F 190	Non-random	Uneven distribution over the day, depending on lobe of origin: Peaks in MTLE and NTLE 1100-1700h; F 2300-0500h and P 1700-2300h

¥ Case report. CPS complex partial seizures; F frontal; I- EEG intracranial electroencephalography; LTLE lesional temporal lobe epilepsy; MTLE mesial temporal lobe epilepsy; NTLE neocortical temporal lobe epilepsy; O occipital; P parietal; S- EEG superficial electroencephalography; TLE temporal lobe epilepsy; XTLE extratemporal lobe epilepsy

The influence of epilepsy on circadian rhythms

In comparison, there is little data on the circadian rhythm in human epilepsy. More is known on the influence of epilepsy and seizures on several circadian rhythm mediated body functions.

Chronotypes

Chronotype refers to the preferred phase of an individual for the timing of daily activities, sleep and wakefulness. Knowledge on the distribution of chronotypes in epilepsy is very poor. In two studies of rodent epilepsy models, daily activity patterns were studied. One study showed marked changes in daily activity patterns for up to 12 weeks after a pilocarpine-induced status epilepticus (Stewart and Leung, 2003). The acrophase (rhythm peak) was delayed for at least 12 weeks. In another study, behavioural rhythms of rodent models of chronic atypical absence seizures were recorded (Stewart et al., 2006). In this study, there was no phase shift, but hyperactivity in the mice was observed.

Pung and Schmitz studied the circadian rhythms of 20 patients with juvenile myoclonic epilepsy (JME) and 20 patients with TLE (Pung and Schmitz, 2006). They found interesting differences in the circadian activity patterns of both groups. It was observed that patients with JME seem to have a characteristic circadian rhythm comparable to an extreme eveningness (going to bed later at night, getting up later in the morning and feeling fit at a later time during the day). Patients with TLE could be defined as ‘morning types’. No other reports have been published on the distribution of chronotypes in patients with epilepsy. Whether certain chronotypes influence epilepsy and timing of seizures is not known.

Sleep and sleep-wake cycle

The interaction of sleep and epilepsy has been studied extensively. For instance, it has been shown that the occurrence of seizures differs in different sleep stages: NREM, especially stage II, facilitates partial seizures, whereas REM sleep is inhibitive for seizures. Also, partial seizures tend to generalize more often during sleep than when awake (Bazil and Walczak, 1997).

Epilepsy and seizures can also have considerable influence on sleep and sleep quality. The effect of recent seizures, anti-epileptic drugs (AEDs) and severity of the epileptic disorder may result in disorganized nocturnal sleep in people with epilepsy. Various epilepsy syndromes and types of seizures can influence sleep to a different extent (Sammaritano and Therrien, 2002). As there are many well written reviews and text books addressing this subject, this will not further be discussed here.

The sleep-wake cycle is a complex process that is determined by the interaction of several factors. According to the two-process model of sleep regulation, timing and structure of sleep are determined by the interaction of the circadian pacemaker (process C), which promotes alertness during the subjective day and sleepiness during the subjective night and a homeostatic increase in sleepiness (process S), which depends on the prior time awake. Other important influences are sleep inertia and body temperature. As the circadian clock is only part of this whole process, great caution should be applied when using the sleep-wake cycle as a synonym for the circadian rhythm in (epilepsy) research.

Core body temperature

Very few studies have considered core body temperature (CBT) rhythms in animals and patients with epilepsy. In rats with electrically induced limbic epilepsy it was shown that the circadian component of the CBT was preserved in the epileptic animals (Quigg et al., 1999). However, the other aspects of the CBT were more complex and the curves were more disordered than those of the control animals. When these epileptic animals were maintained in constant darkness, with a free running circadian rhythm, the temperature curves became more complex and even polyrhythmic. Isolated acute seizures had no effect on the ultradian rhythms in CBT in these animals. In a more recent study, changes in CBT after seizures were found (Quigg et al., 2001). Postictal phase shifts in CBT and a more complex CBT rhythm were also observed.

In one study, oral temperature rhythms were measured in patients with TLE (Bazil et al., 1999). Twenty-four hour periods after a seizure were compared with a seizure free 24-h period. The temperature patterns remained the same. The interpretation of these findings, however, is difficult as no healthy controls were included in this study.

Body temperature can also affect seizures, e.g. fever can act as a trigger to induce seizures. Conversely, data from epileptic rats show that body temperature recorded in ten minute epochs during which seizures occurred, was slightly lower than in epochs during which no seizures were recorded (Quigg et al., 2000). This data supports the hypothesis that normal, non-pathological elevations in CBT are not associated with higher seizure occurrence.

Circadian rhythms in cardiovascular parameters

Several cardiovascular parameters are under the influence of the circadian clock. For instance, there is a clear 24-h rhythm in blood pressure (BP) and heart rate variability (HRV). Altered cardiovascular autonomic regulatory functions in epilepsy could be involved in the pathogenesis of sudden unexpected death in epilepsy (SUDEP). Therefore, it is not surprising that numerous groups have studied cardiovascular parameters in relation to epilepsy (table 2). Several well-conducted studies have found reduced circadian HRV in people with chronic epilepsy (Ansakorpi et al., 2000; Ansakorpi et al., 2002; Isojarvi et al., 1998; Persson et al., 2005; Persson et al., 2006; Persson et al., 2007b; Ronkainen et al., 2005; Tomson et al., 1998). One study showed greater variability in BP and heart rate during testing of the autonomic nervous system in patients with epilepsy on carbamazepine (CBZ) treatment (Devinsky et al., 1994).

It is important to realise that most studies have included patients with TLE and that these results cannot be extrapolated to people with epilepsy in general. Furthermore, almost all groups have included patients receiving AEDs, mainly CBZ. The influence of the disease itself and that of AEDs cannot be differentiated with certainty. In a few studies no effect on HRV in newly diagnosed, untreated patients could be recorded (Isojarvi et al., 1998; Persson et al., 2007a). Evrengul et al. found increased sympathetic control of HRV in young males with unclassified untreated epilepsy. In patients with juvenile myoclonic epilepsy no difference was found in HRV between patients and controls (Evrengul et al., 2005; Tomson et al., 1998).

The precise influence of different AEDs on cardiovascular parameters is the subject of various other studies and is beyond the scope of this review. The effects of ictal and interictal

discharges on cardiovascular parameters have been studied and reviewed extensively elsewhere (see Baumgartner et al., 2001).

Melatonin

Melatonin has been studied intensively with respect to epilepsy and seizures. In several animal studies it has been shown that melatonin has anticonvulsant effects against electrically-induced seizures (Lapin et al., 1998; Mevissen and Ebert, 1998). Also, removing the pineal gland leads to seizure activity, which can be counteracted by the administration of exogenous melatonin (Rudeen et al., 1980).

In humans the effect of epilepsy on melatonin and vice versa has been described in several studies. Results, however, are conflicting (table 3). Some authors describe low baseline levels of melatonin in people with epilepsy (Bazil et al., 2000; Fauteck et al., 1999; Laakso et al., 1993; Yalyn et al., 2006), whilst others found elevated levels (Molina-Carballo et al., 1994; Schapel et al., 1995). Likewise, elevated levels during or directly after complex partial seizures (CPS) and generalized tonic clonic seizures (GTCS) have been described (Bazil et al., 2000; Molina-Carballo et al., 2007), whilst other authors observed no changes after CPS and GTCS (Rao et al., 1989).

The effect of melatonin on seizure frequency has also been studied. It has been found that administration of melatonin to people with epilepsy can prevent seizures (Fauteck et al., 1999; Molina-Carballo et al., 1997; Peled et al., 2001). With discontinuation of melatonin, seizure rates increased to previous levels (Molina-Carballo et al., 1997). In other studies, however, it was found that melatonin had no clear group effect on seizure frequency or was even associated with increased seizure activity (Coppola et al., 2004; Sheldon, 1998).

Cortisol, prolactin and growth hormone

Many studies have been conducted on human cortisol levels in epilepsy. No difference in baseline levels of cortisol between people with epilepsy and controls has been found. Postictal elevations of cortisol levels have been described after almost all GTCS, most CPS and some simple partial seizures. Only two studies have described a pre-ictal level and found a low cortisol.

Prolactin levels in relation to human epilepsy and seizures have also been the focus of intense research. It can be concluded from these studies that baseline levels are higher than in normal controls and that prolactin may rise after seizures. Psychogenic seizures have no influence and therefore the level of prolactin may be used to differentiate between epileptic and psychogenic seizures.

Growth hormone has also been studied. Studies disagree as to whether the level of growth hormone in humans rises after GTCS and CPS, but most studies have observed a rise after most GTCS and some CPS. For extensive reviews on these hormones in epilepsy, see Pritchard and Bauer (Bauer, 1996; Pritchard, III, 1991).

Table 2. Studies on cardiovascular parameters in patients with epilepsy

Reference	Pathology (*n*=)	Intervention	Methods	Results
(Frysinger et al., 1993)	Complex partial seizures (19)	-	24 hour ECG	Reduced HRV in TLE
(Devinsky et al., 1994)	Partial seizures, receiving CBZ (24)	Diverse	ECG and BP	Greater BP variability, HRV and reactivity in epilepsy patients than controls
(Tomson et al., 1998)	JME (21) TLE (21)	-	24 hour ECG	Reduced HRV in patients with TLE; No reduced HRV in JME patients
(Isojarvi et al., 1998)	Untreated epilepsy (37) Treated chronic epilepsy (47)	Diverse	ECG and BP	No effect in newly diagnosed. Diminished cardiovascular responses in chronic epilepsy
(Ansakorpi et al., 2000)	Refractory TLE (19) Controlled TLE (19)	Tilting	ECG and BP	Reduced HRV in refractory TLE and controlled TLE
(Ansakorpi et al., 2002)	Refractory TLE (19) Controlled TLE (25)	-	24 hour ECG	Reduced HRV in TLE
(Persson et al., 2005)	TLE (21)	Epilepsy surgery	24 hour ECG	More pronounced impairment of S and PS cardiac control in patients with poor surgery outcome
(Evrengul et al., 2005)	Unclassified untreated epilepsy (43)	-	ECG	Increased sympathetic control of HRV
(Ronkainen et al., 2005)	Refractory TLE (17) Controlled TLE (20)	-	24 hour ECG	Reduced HRV, more pronounced during night than day
(Persson et al., 2006)	TLE (21)	Epilepsy surgery	24 hour ECG	No effect of surgery on reduced HRV
(Persson et al., 2007b)	Newly diagnosed epilepsy (14) TLE (21)	Start CBZ therapy or surgery	24 hour ECG	Reduced HRV may be more pronounced during the night
(Persson et al., 2007a)	Newly diagnosed untreated epilepsy (22)	-	24 hour ECG	No effect on HRV in untreated newly diagnosed epilepsy patients

AED anti-epileptic drugs; BP blood pressure; HRV heart rate variability; JME juvenile myoclonic epilepsy; PS parasympathetic; S sympathetic; TLE temporal lobe epilepsy; yr year

Table 3. Studies on melatonin in epilepsy and seizures

Reference	Pathology (*n*=)	Intervention	Methods	Results
(Rao et al., 1989)	6	-	Postictal serum MLT levels	No alterations in MLT levels during or after seizures
(Laakso et al., 1993)	LGS (16)	-	Saliva MLT levels every 2 h	Six of 16 patients were low-secretors compared to controls
(Molina-Carballo et al., 1994)	28	-	Postictal serum MLT levels (1-3 h)	Higher baseline level of MLT with a maintained day-night rhythm
(Schapel et al., 1995)	Active untreated epilepsy (30)	-	Urinary excretion of MLT metabolite (3x/ day)	Increased MLT levels and a circadian pattern with a phase difference compared to controls
(Molina-Carballo et al., 1997)	Progressive myoclonic epilepsy (1)	40-500 mg MLT a day	Seizure frequency	Decrease of seizure rate from 15-20/ day to zero. Return to former seizure rate after discontinuing the drug
(Sheldon, 1998)	Generalized epilepsy (5 children)	5 mg MLT at habitual bedtime	?	Increased seizure activity in four children
(Fauteck et al., 1999)	Severe epilepsy (10 children)	5-10mg MLT before bedtime	Seizure diary; Subjective sleep rating	Low MLT levels in six children; Decreased seizure frequency in six; Improved sleep in eight
(Bazil et al., 2000)	TLE, no medication (11)	-	MLT levels in saliva (8x/ day)	Low MLT levels compared to controls. Postictal increase
(Peled et al., 2001)	Severe intractable epilepsy (6 children)	3 mg MLT 30 min before bedtime	Seizure diary+ PSG (n=3)	Significant clinical improvement in 5 children
(Coppola et al., 2004)	Mental retardation and epilepsy (18)	3-9 mg MLT at nocturnal bedtime	Seizure diary	Poor influence of MLT on seizure frequency
(Yalyn et al., 2006)	CP epilepsy (20)	-	Serum MLT levels (4x/day)	Low MLT levels compared to controls
(Molina-Carballo et al., 2007)	Convulsive crisis, febrile and epileptic (54)	-	Serum MLT levels during seizure + 1 and 24 h afterwards	Significant increase of MLT during seizure, normal postictal values after 1 and 24 h

AED anti-epileptic drugs; CP complex partial; h hour; LGS Lennox Gastaut Syndrome; MLT melatonin; PSG polysomnography; TLE temporal lobe epilepsy

Summary and Discussion

Several studies have focused on the interaction between circadian rhythms and (human) epilepsy. Seizure occurrence seems to have 24-h rhythmicity, depending on the lobe of origin. An important finding in one rodent model of limbic epilepsy was that a true endogenous mediated circadian rhythm in seizure occurrence was shown when the animals were placed in constant darkness. In interictal discharges it has mainly been the influence of sleep and wake that is studied and not the course over the 24-h day. No data has been published (yet) on clock gene profiles in epilepsy patients. More is known about the influence of human epilepsy and seizures on circadian rhythms. One study has focused on chronotypes and found significant differences in chronotypes between these two groups of patients with epilepsy (TLE and JME). Numerous studies have focused on sleep in epilepsy and describe clear influences of epilepsy and seizures on sleep. In contrast, knowledge about core body temperature in people with epilepsy is minimal. When studying circadian rhythm mediated cardiovascular parameters, reduced heart rate variability in people with chronic epilepsy has been shown by several authors. Finally, several hormones have also been studied. Results on the interaction of melatonin and epilepsy are not conclusive, as differences in baseline levels and postictal levels have been found. Normal baseline levels of cortisol have been described with postictal elevations. Elevated baseline prolactin levels have been observed and levels may rise further after seizures. Studies on growth hormone disagree, but most studies have observed a postictal increase.

Even though many questions remain, it can be concluded from the studies described above, that there is proof that circadian rhythm and epilepsy at least interact. Unfortunately, there are considerable gaps in the knowledge of this interaction, especially in humans. All human studies are very informative, but these have been performed in daily life. This means these subjects are entrained by Zeitgebers and true endogenous circadian patterns cannot be observed. To explore the endogenous rhythm, constant routines trials are required (Duffy and Dijk, 2002). These trials have been performed frequently in healthy subjects, but it is ethically challenging to perform such trials in epilepsy patients as sleep deprivation enhances seizures. In animal studies, it is easier to explore true circadian rhythms as has been shown by Quigg et al. (Quigg et al., 2000). More elaborate trials with animal epilepsy models could be performed, including phase shifting of the circadian rhythm and applying different AEDs at different times in the circadian rhythm, to study the effects on seizure frequency and temporal distribution of seizures. Of interest in humans would be to study correlation between the occurrence of seizures and the individual circadian rhythm. Also, a more extensive study of the distribution of chronotypes in various epilepsy syndromes would be informative. Furthermore, adjustment of anti-epileptic treatment to the individual circadian rhythm may improve control and deserves to be studied.

Several of these topics will be studied in our epilepsy centre and collaborating centres, as more and thorough research is warranted to further explore the interaction between the circadian rhythm and epilepsy. As mentioned, this may be of high value for better knowledge of pathophysiology, timing of diagnostic procedures and therapeutic options in epilepsy.

Chapter Three

How to assess circadian rhythm in humans: A review of literature

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Epilepsy & Behavior (2008) 438-444

Abstract

It is well known that seizures of some types of epilepsy tend to occur in patterns. The circadian rhythm may play a significant role in this phenomenon. In animal studies it has been found that seizures in experimental partial epilepsy are probably under the influence of the biological clock. In this review an introduction to the influence of the human circadian rhythm in epilepsy is given. Furthermore, the methodology of measuring the circadian rhythm in humans is explored. An overview of widely used methods includes protocols used to desynchronize circadian rhythm and sleep-wake cycle and biological markers such as the dim light melatonin onset, core body temperature and cortisol that are employed to determine the phase of the circadian rhythm. Finally, the use of sleep parameters, actigraphy and questionnaires is discussed. These are also important in assessment of the circadian rhythm.

Introduction

It is well known that the occurrence of seizures is not entirely random. This was first described in 1885 by Gowers, who classified patients into three groups based on the distribution of ‘‘fits” over the day: diurnal, nocturnal and diffuse (Gowers, 1885). Later studies confirmed and extended his findings. It was observed how diurnal seizures cluster at certain times of the day, namely, on awakening and in the late afternoon and how nocturnal seizures tend to occur mainly at bedtime and the hours before awakening (Griffiths and Fox, 1938; Langdon-Down and Brain, 1929). Also, in more recent studies, it has been confirmed that seizures may occur in patterns depending on the pathophysiology of the epileptic syndrome (Milton et al., 1987; Quigg et al., 1998b; Tauboll et al., 1991). The reason why seizures do so is not well understood. One of the hypotheses is that the circadian clock plays a significant role. The interaction of epilepsy and the circadian rhythm has been studied; however, still very little is known. For instance, Pung and Schmitz compared the chronotypes of patients with juvenile myoclonic epilepsy (JME) with those of patients with temporal lobe epilepsy (TLE). Patients with JME seem to have a characteristic circadian rhythm, with the tendency to go to bed later at night, to get up later in the morning and to feel fit at a later time during the day compared with patients with TLE (Pung and Schmitz, 2006). Another study in a small group of 16 patients with Lennox-Gastaut syndrome demonstrated that disorders of the circadian rhythm were observed in more than half of these patients (Laakso et al., 1993).

In animal studies, several protocols can be used to uncover the circadian rhythm and study a possible interaction in more detail. In a very illustrative study by Quigg (Quigg et al., 2000), epileptic rats were monitored with constant EEG registration. The rats were entrained to a 12 hour-12 hour light-dark cycle and then exposed to constant darkness. Results indicated that during light-dark exposure, spontaneous limbic seizures occurred in statistically non-uniform patterns, with nearly twice as many seizures during the light portion. During the period of constant darkness, seizures continued to occur in the same pattern observed during light-dark exposure when referenced to the circadian rhythm of these rats, suggesting that spontaneous limbic seizures recur in a true endogenously mediated circadian pattern.

More research is required to elucidate the possible interaction between the circadian rhythm and epilepsy. If this interaction indeed exists, it has important diagnostic and therapeutic consequences, for example, improved control over epilepsy through administration of anti-epileptic medication according to an individual’s circadian rhythm.

Circadian rhythm

The mammalian biological clock consists of a hierarchy of oscillators, the master circadian pacemaker of which is found in the brain, formed by the cells of the suprachiasmatic nuclei (SCN) within the anterior hypothalamus. The pacemaker in these nuclei generates and maintains circadian rhythms in many physiological and psychological processes, including the sleep-wake cycle, core body temperature, blood pressure, task performance and synthesis and secretion of several hormones, such as melatonin and cortisol (Hastings et al., 2007). The intrinsic period of the human biological clock is ~24.2 hours, with surprisingly small inter- and intraindividual ranges (Czeisler et al., 1999). This period is slightly longer than our 24-h day, hence the name circadian (circa meaning ‘‘around,” and dies meaning

‘‘day”) rhythm. As in every rhythm, important features of the circadian clock are phase, amplitude and period.

To synchronize the circadian system to the exact 24-h sleep-wake cycle, the SCN need to adjust daily. This is termed entrainment and is one of the key characteristics of the biological clock. Entrainment is accomplished by external cues, the so-called Zeitgebers (‘‘time givers” in German). Examples of Zeitgebers are scheduled sleep, activity, temperature and social external signals such as the clock and meals. However, by far the most important Zeitgeber is light or the solar light-dark cycle (Duffy and Wright, Jr., 2005). As light enters the eye, it is picked up by specialized photoreceptors in the ganglion cells of the retina (Berson et al., 2002). The signal travels from the retina to the SCN via a neuroanatomical pathway called the retinohypothalamic tract and resets the SCN. The extent of this resetting response depends on the wavelength, intensity, timing, number, pattern and duration of exposure to light (Duffy and Wright, Jr., 2005).

Circadian output

The SCN exert their influence via projections throughout the hypothalamus, thalamus and limbic system (Buijs and Kalsbeek, 2001). One of the main target organs of the SCN is the pineal body. An important task of this small brain structure is the synthesis of melatonin from tryptophan. This production is highly rhythmic in all mammals examined thus far. Its rhythm is characterized by a low daytime level, ascending after the onset of darkness to high output during the night (with its peak between 2300 and 0300h) and then falling sharply before the onset of light (Figure 1A). The secretion of melatonin continues in a near-square-wave pattern that continues even in constant darkness (Ralph et al., 1971). The activity of the SCN and the secretion of melatonin are two of the rare circadian rhythms occurring similarly in nocturnal and diurnal species. This similar rhythm in diurnal and nocturnal mammals is due to the suppression of synthesis of this hormone by light (Lewy et al., 1980).

Apart from the master circadian pacemaker in the SCN, there is convincing evidence for the existence of peripheral circadian oscillators in the human body. More or less independent peripheral oscillators are found in several organs, including liver, skeletal muscle and testis, all under the influence of the SCN (Plautz et al., 1997; Zylka et al., 1998).

Interindividual differences

Circadian rhythmicity has been demonstrated in several processes, such as core body temperature (CBT) and hormone secretion. The phases of these rhythms vary between individuals. This has been attributed to factors such as gender, age and especially morningness/eveningness, which is reflected in the individual’s preference for sleep-wake timing (Vink et al., 2001). ‘‘Morningness” scores indeed correlate with the timing of the individual’s circadian pacemaker (Duffy et al., 2001). In comparison to evening types, morning types tend to schedule sleep earlier and experience earlier peaks of alertness and performance during the day (Andrade et al., 1992) and markers of circadian rhythm (such as CBT, melatonin and cortisol) show an earlier phase in morning types as compared with evening types (Kerkhof and Van Dongen, 1996) (Duffy et al., 1999). However, morning and evening types paradoxically do not go to bed and wake up at the same circadian time (Duffy

et al., 1999). Here, we define circadian as the time point in the individual's circadian rhythm according to the sleep midpoint (sleep onset time - wake up time/2) of that individual.

Several genes have been discovered that are, at least partly, responsible for this characteristic activity of the individual SCN and the interindividual differences (Cermakian and Boivin, 2003). The activity depends on the expression of autoregulatory translation-transcription feedback loops of genes including the Period genes (Per1, Per2, Per3), the Clock gene and two Cryptochrome genes (Cry 1, Cry2) (Van Gelder et al., 2003). It has been demonstrated in several animal studies that deletion or mutation of these genes leads to rhythms with abnormal periods or even arrhythmic phenotypes when tested under constant conditions (Cermakian and Boivin, 2003; Ko and Takahashi, 2006). With this knowledge, it is no surprise that many researchers are trying to establish a genetic basis for circadian rhythm disorders. This has been successful to a certain extent in a few circadian rhythm disorders (Toh et al., 2001; Xu et al., 2005). Moreover, dysfunction of these clock genes might be important in the development of various diseases, including cancer (Lamont et al., 2007).

In this review, we describe methods frequently used to measure circadian rhythm in humans. Protocols are discussed that can be used in research settings and are even necessary in certain situations to ensure collection of pure data. In addition, widely used biological phase markers are reviewed and sleep parameters, questionnaires and actigraphy are also addressed.

How to measure the circadian rhythm

Protocols

To study the underlying periodicity of the biological clock, it is necessary to rule out all influencing external factors (so-called masking factors). In this way, internal time can be desynchronized from external time. The first to conduct such desynchronizing experiments was Nathaniel Kleitman. In 1938, he scheduled subjects to live on artificial day lengths in Mammoth Cave in Kentucky (Cajochen et al., 2006). Under these conditions, the circadian clock of these subjects could not entrain and continued to oscillate with their own endogenous periods (i.e. "free running"). Later on, many researchers used the same principle to study the circadian rhythm. For instance, in 1995, Dijk and others scheduled subjects to a 28-h sleep-wake cycle, resulting in sleep episodes at all phases of the endogenous circadian cycle. Circadian and sleep-wake components could be distinguished very well in this way (Dijk and Czeisler, 1995). However, this protocol is very labour-intensive and long-lasting, as it takes at least 4 weeks to complete. Therefore, Hiddinga and others used a different protocol lasting 120 hours to study body temperature in humans (Hiddinga et al., 1997). This protocol, consisting of six 20-h days, demonstrated that a shorter forced desynchrony protocol also succeeds in reliable differentiation of the endogenous circadian rhythm.

Besides the forced desynchrony protocols, the ''constant routine" protocol has been developed to reveal unmasked circadian rhythms as well (Mills et al., 1978). The constant routine protocol minimizes or eliminates the external factors that are known to obscure the endogenous component of circadian rhythms by keeping these factors as constant as possible. This means that the patient is kept awake in a semi recumbent position and is supplied equally distributed small meals for at least 24 hours in a room with constant temperature and humidity and in dim light conditions (Duffy and Dijk, 2002). Although this

constant routine protocol can be very useful in many studies, it does not desynchronize the sleep-wake cycle from the circadian pacemaker. Moreover, as sleep is also a masking factor, subjects should be kept awake during the entire constant routine. The resulting sleep deprivation may unfortunately influence the interpretation of results or modify the circadian patterns studied and therefore, this protocol cannot be used in studies of diseases influenced by sleep deprivation, for example, epilepsy (Hiddinga et al., 1997). To avoid the accumulation of sleep pressure, the multiple-nap protocol has been designed. This is a constant routine protocol with multiple longer naps scheduled over a 24-h day or longer (Cajochen et al., 2001). These naps prevent the accumulation of sleep pressure; thus, an important masking factor is strongly reduced and the circadian rhythm emerges very clearly. Moreover, this is a short protocol in comparison with the other long-lasting forced desynchrony protocols.

Finally, the circadian rhythm can also be shifted by exposure to bright light in cases of fixed timing of the sleep-wake cycle. The magnitude and direction of the phase shift depend on the phase of the rhythm at the time of light exposure. Light early in the subjective night causes delay to a later phase and light late in the subjective night causes advances to an earlier phase (Voultsios et al., 1997).

Markers of circadian rhythm

In theory, all output rhythms driven by the biological clock that can be measured or evaluated can be used to assess the phase, period and amplitude of the circadian rhythm. However, in practice, the most widely used measures are melatonin, core body temperature and cortisol production.

Dim light melatonin onset

Levels of melatonin can be measured in plasma or saliva (with a concentration that is approximately three times lower than that in serum (Voultsios et al., 1997)) and levels of its metabolite, 6-sulfatoxymelatonin, in urine. Measurement of the entire 24-h rhythm of melatonin is considered to be the most robust phase marker (Van Someren and Nagtegaal, 2007). However, as this is time-consuming and also inconvenient for the subject, this method is not frequently employed. More often, the moment at which melatonin production starts rising in the evening (the onset) is used as an indication of the circadian phase. However, ambient light intensities as encountered outdoors (i.e. 3000-100.000 lx) suppress the production of melatonin and some studies report that even as little as ~100-180 lx (room light intensity) can produce phase shifts in the circadian rhythm (Boivin and Czeisler, 1998; Zeitzer et al., 2000). The degree of suppression seems to depend on the light history, that is, to what intensity of light the subject was exposed prior to the study (Hebert et al., 2002; Smith et al., 2004). To avoid erroneous values, melatonin samples should preferably be taken in dim light (<50 lx). Therefore, this procedure is termed the dim light melatonin onset (DLMO) (Benloucif et al., 2008). As noted, the DLMO reflects the phase of the circadian rhythm and, if measured over more than one cycle, also the period of the endogenous circadian pacemaker. The DLMO can be observed about 2 to 3 hours before habitual bedtime (1930-2200h), if the individual's phase is approximately normal (Lewy et al., 1999).

Determining the DLMO from a partial melatonin profile (i.e., not over the entire 24 hours) can be done in several ways. An absolute threshold can be taken, for example, in the range of 2 to 10 pg/ml in serum. Although intraindividual differences in melatonin profiles are

small, there are large differences in absolute production of melatonin between individuals. Some people produce very little melatonin (low producers) and in these cases, using the threshold of 2 pg/ml is recommended (Benloucif et al., 2008). In saliva, an absolute threshold of 3 pg/ml can be used (Lewy et al., 1999). To obtain more reliable values in these situations, mid sleep melatonin measurement can be performed to delineate the peak melatonin value. A second way of determining the DLMO is by calculation of a threshold at 2 SD above the average baseline samples (at least three samples needed prior to DLMO measurement) (Duffy et al., 2001). Also, a visual estimate can be made of the point of change (baseline to rising).

The DLMO is considered to be one of the most reliable markers, as it is thought to be minimally masked by exogenous factors. There is, however, some evidence that several factors can mask the melatonin level to some degree. These include posture, exercise, sleep and sleep deprivation, caffeine and certain drugs, for instance, NSAIDS and beta blockers (Deacon and Arendt, 1994; Monteleone et al., 1990; Murphy et al., 1996; Shilo et al., 2002; Stoschitzky et al., 1999; Zeitzer et al., 2007). When the DLMO is used as a marker, it is important to control these factors as much as possible to obtain reliable values.

Countless studies have tried to determine the relationship between age and secretion of melatonin. However, the results of these studies are not consistent on whether this secretion is age related and whether melatonin levels decrease with increasing age (Munch et al., 2005; Zeitzer et al., 1999). It remains to be elucidated whether this age-related reduction exists.

Melatonin is an intensively studied hormone with respect to epilepsy and epileptic seizures. Its precise value is still disputed, as results are very inconsistent. Melatonin has been shown to have a depressive effect on brain excitability and to prevent seizures in several animal models (Champney et al., 1996; Lapin et al., 1998). However, Sandyk et al. reported that it can also have a proconvulsive effect in humans (Sandyk et al., 1992). Some authors have described low levels of melatonin in patients with epilepsy, whereas others have measured normal or elevated levels (Bazil et al., 2000; Laakso et al., 1993; Rao et al., 1989; Schapel et al., 1995). Likewise, in some studies levels were elevated after complex partial seizures, whereas in other studies, no changes were observed after complex partial and generalized tonic-clonic seizures (Bazil et al., 2000; Rao et al., 1989). Further research is necessary to define the precise effects of epileptic seizures on melatonin and its role in seizure prevention and epilepsy.

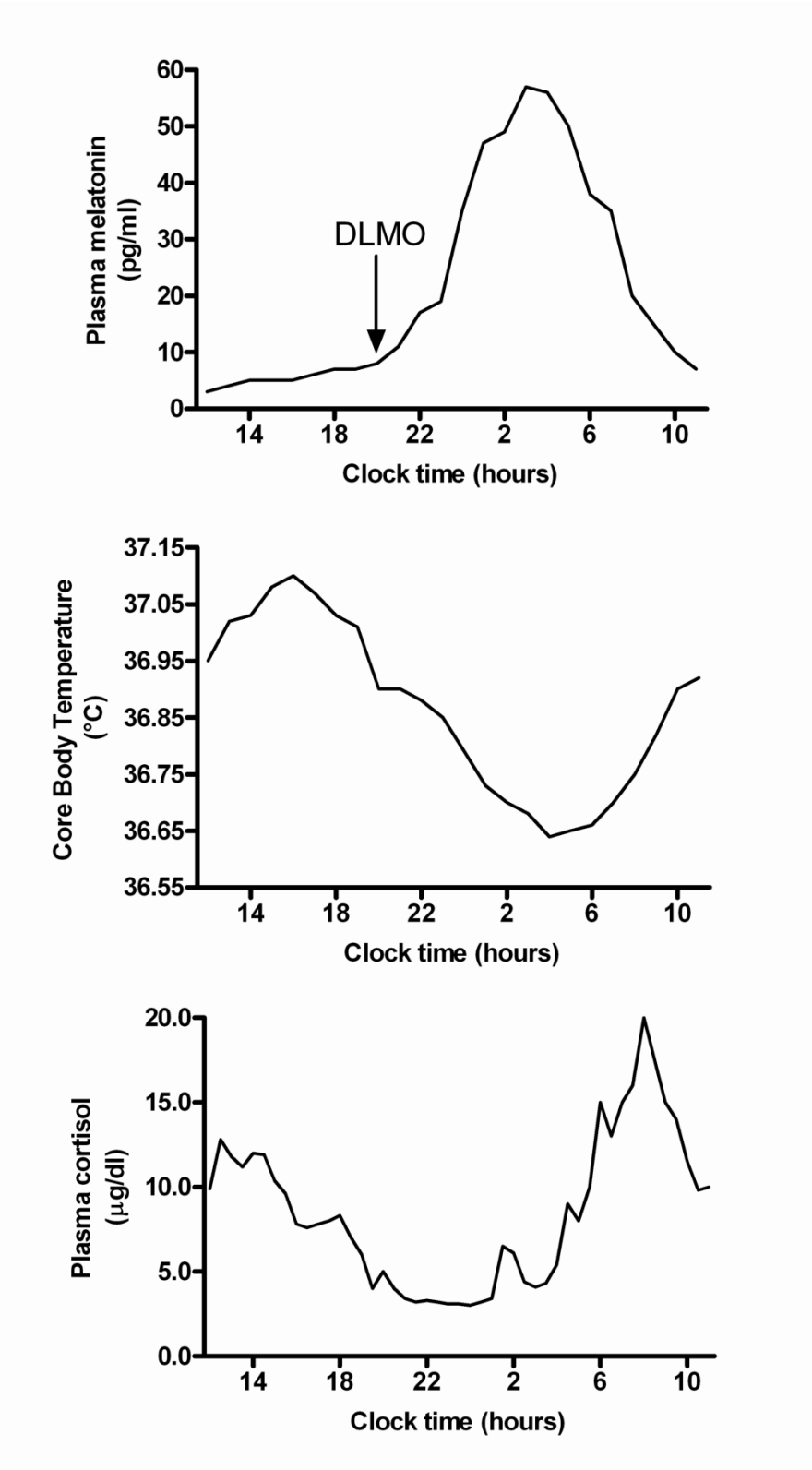

Figure 1. Human circadian rhythms of the plasma melatonin level (A), core body temperature (B) and plasma cortisol level (C). Dim light melatonin onset (DLMO) is indicated in (A).

Core body temperature

Core body temperature (CBT) was first described to possess a circadian rhythm by Gierse in Germany in 1842. Gierse showed that his own oral temperature reached a maximum in the early evening and a minimum in the early morning (Waterhouse et al., 2005). Accumulating evidence since then has confirmed this rhythm and has demonstrated that CBT is indeed one of the physiological processes regulated by the circadian pacemaker (Krauchi, 2002).

The circadian rhythm of the CBT is determined by the combined action of heat production and heat loss. When heat loss exceeds heat production, CBT declines and vice versa (Waterhouse et al., 2005). This rhythm is characterized by a nocturnal decline, which is a consequence of greater heat loss and vasodilatation at distal skin regions (Krauchi et al., 1997). In humans, sleep is typically initiated when this curve is declining at its maximum rate (Campbell and Broughton, 1994). After reaching its minimum, the so-called nadir (reached at approximately 0500h), CBT increases and reaches its maximum (at approximately 1700h) during the day, because then heat production surpasses heat loss (Figure 1B).

As it is easy to collect continuous data from a subject without much disturbance and data can be analysed immediately, CBT is also a frequently used marker of the central circadian rhythm. However, many factors can influence CBT, masking the true endogenous signal. These factors include behaviours such as postural changes, physical activity and meals and also external conditions, such as ambient temperature, sound, humidity and bright light (Ancoli-Israel et al., 2003; Dauncey and Bingham, 1983; Dijk et al., 1991; Gander et al., 1986; Krauchi et al., 1997; Krauchi et al., 2006; Moran et al., 1995). Earlier studies also hypothesized that sleep influences CBT; however, more recent studies contradict these conclusions (Krauchi and Wirz-Justice, 2001). Age also influences the rhythm. For example, in the elderly, CBT reaches its nadir earlier (Czeisler et al., 1992). In their study, Quigg et al. reported that the circadian rhythm of CBT in epileptic rats (models for mesial TLE) is more complex and polyphasic than that in normal rats (Quigg et al., 1998a). However, acute stimulated seizures did not affect this complexity.

The constant routine protocol is, as described earlier, designed to minimize the influence of masking factors by keeping these as constant as possible. Another way of minimizing masking effects on CBT is through the use of mathematical adjustments of the temperature rhythm (Waterhouse et al., 2000). A third way is using a forced desynchrony protocol to distinguish the variable caused by masking factors from that related to the circadian pacemaker (Dijk and Czeisler, 1995; Hiddinga et al., 1997).

Cortisol

Cortisol is a corticosteroid hormone that is produced by the zona fasciculata of the adrenal cortex. The endogenous pacemaker also generates a circadian rhythm in this hypothalamic-pituitary-adrenal secretion via a multi-synaptic suprachiasmatic nucleus-adrenal pathway (Buijs et al., 1999). Cortisol secretion is therefore highly rhythmic, with declining levels throughout the day, a nocturnal period of quiescence and a sharp rise in the second half of the night toward a morning maximum (the acrophase), particularly in the early morning, some hours before and just after waking up (Figure 1C). The amplitude of the secretory episodes declines throughout the morning and is minimal in the evening. The nadir is reached within approximately 2 hours after beginning sleep (Veldhuis et al., 1990).

Cortisol levels can be measured in serum and saliva. Serum free cortisol diffuses freely into saliva and measurements of salivary cortisol reflect serum free cortisol concentrations more accurately than measurements of total cortisol in the serum (Umeda et al., 1981).

Several different characteristics of the cortisol rhythm can be used as markers of circadian rhythm, including the timing of the nadir or acrophase, the onset of the evening rise and the start or end of the quiescent period (Van Cauter et al., 1996; Weibel and Brandenberger, 2002). However, several factors influencing secretion of cortisol are known. Physical and physiological stress also activates the hypothalamic-pituitary-adrenal axis, which results in bursts of secretory activity. Light is another influencing factor, as it raises the morning cortisol peak significantly and can cause phase shifts (Benloucif et al., 2008; Leproult et al., 2001; Scheer and Buijs, 1999). Furthermore, aging also has its effects on the cortisol rhythm. With increasing age, the cortisol rhythm shifts, as the nadir and maximum of the curve are reached earlier and circadian amplitude is also reduced in elderly subjects (Van et al., 1996; van et al., 1991). The sleep-wake cycle can influence the cortisol level in several ways. Sleep onset and deep sleep inhibit or reduce cortisol secretion, whereas sleep loss, light sleep and awakenings result in elevation of cortisol levels (Caufriez et al., 2002; Gronfier et al., 1998). Consumption of high-protein meals can cause additional secretory episodes (Slag et al., 1981). Several studies describe postictal elevations of cortisol levels (Culebras et al., 1987; Mehta et al., 1994; Rao et al., 1989). However, these changes were not observed in all studies (Molaie et al., 1987). Again, it is necessary to keep the influencing factors as minimal or constant as possible when using this method to measure circadian rhythm.

Apart from melatonin and cortisol, there are a few other hormones that are secreted in a circadian rhythm and are known to be relevant in epilepsy. Noteworthy among these are growth hormone and prolactin. Growth hormone is released in a diurnal pattern with several large pulses each day, a pattern changing throughout life. Studies disagree over whether growth hormone levels rise after generalized tonic-clonic and complex partial seizures (Culebras et al., 1987; Gallagher et al., 1987; Rao et al., 1989). Prolactin secretion is associated with both a diurnal cycle and an ovulatory cycle. It has been shown that nocturnal prolactin rises significantly after generalized tonic-clonic or complex partial seizures (Kurlemann et al., 1992; Molaie et al., 1987; Valdizan et al., 1992). Therefore, the level of prolactin has been proposed as a method to differentiate between epileptic and psychogenic seizures (Rao et al., 1989).

Combinations and correlations

Under conditions of a constant routine, the correlation between CBT and DLMO as phase markers is usually high (Sack et al., 2007; Shanahan and Czeisler, 1991). Therefore, the combination of these two markers is frequently used in studies. DLMO and cortisol levels also constitute a well-known combination in circadian studies. For instance, Weibel et al. compared cortisol levels and the DLMO in a group of shift workers and found that the start of the quiescent period remained phase-locked to the DLMO (Weibel and Brandenberger, 2002).

In a recent study, Klerman et al. compared the variability of all three markers discussed above. They concluded that methods using plasma melatonin as a marker may be considered more reliable than methods using CBT or cortisol as an indicator of circadian phase in humans (Klerman et al., 2002). Taken together, the DLMO is the most widely used

phase marker of these three, as the data are easy to collect and easy to interpret, masking is relatively low and masking factors can be controlled fairly simply.

Sleep parameters

The sleep-wake cycle is a complex process that is determined by interaction of several factors. According to the two-process model of sleep regulation, timing and structure of sleep are determined by the interaction of the circadian pacemaker (process C), which promotes alertness during the subjective day and sleepiness during the subjective night and a homeostatic increase in sleepiness (process S), which depends on the prior time awake (Borbely, 1982; Daan et al., 1984). Other important players in this field are sleep inertia and temperature (Krauchi et al., 2005). As the circadian clock is only one participant in the whole process, only a few sleep parameters are useful as phase markers of the circadian rhythm. The most reliable phase marker with respect to sleep timing is the sleep midpoint (Wirz-Justice, 2007). For instance, studies have shown that the DLMO significantly correlates with sleep onset, sleep midpoint and wake time in normal healthy young adults, but most significantly with sleep midpoint (Martin and Eastman, 2002). Furthermore, the DLMO can be readily estimated in people whose sleep times are minimally influenced by family commitments, school, or work (‘‘free” sleepers). On the other hand, this cannot be done in people whose habitual bedtimes are ‘‘fixed” by external commitments, for example, because they have to get up early in the morning to go to work (Burgess and Eastman, 2005).

In short, certain sleep parameters are being used in human circadian studies, but fall short in comparison to the other methods reviewed in this article.

Questionnaires

Several different questionnaires have been developed to study individual aspects of the timing of daily activities and sleep. The Morningness Eveningness Questionnaire (MEQ) was developed by Horne and Ostberg in 1976 (Horne and Ostberg, 1976). This most widely used questionnaire differentiates morning and evening types and this, as explained earlier, does reflect some part of the circadian rhythm. The MEQ contains 19 questions, most of which elicit preferences in timing of daily activities and sleep. This questionnaire is also frequently used to investigate the correlation between these morningness/eveningness preferences (phenotypes) and genotypes (Katzenberg et al., 1998; Vink et al., 2001). A recent, very large review concluded that overall, the MEQ appears to be a fair predictor of the endogenous circadian phase or period (Sack et al., 2007).

In attempts to improve the MEQ and design questionnaires aimed at more specific groups or situations, several other questionnaires have since been developed. The Circadian Type Questionnaire (CTQ) was originally developed to identify which individuals adjust readily to shift work. Since then, this questionnaire has been revised into the Circadian Type Inventory (CTI); very few studies on the correlation between circadian rhythm and this inventory have been published (Baehr et al., 2000). The Diurnal Type Scale (DTS) of Torsvall and Åkerstedt (1980) was also developed for use with shift workers (Torsvall and Akerstedt, 1980). In 1989 Smith et al. proposed an improved morningness scale (the Composite Scale of Morningness) composed of items from the MEQ and DTS (Smith et al., 1989). Finally, the Munich Chronotype Questionnaire (MCTQ) by Roenneberg (2003) was developed to determine the chronotype in a general population, just like the MEQ (Roenneberg et al., 2003). In contrast to the MEQ, the MCTQ explicitly assesses working

and free days separately, as sleep timing can be fairly different on these days. Also, the time spent outside during the day is assessed, to correct for these factors when using timing of sleep as an indicator of the underlying chronotype. Furthermore, the MEQ contains mostly preferential questions, whereas the MCTQ collects information about the actual timing of daily sleep and activities (Zavada et al., 2005). Despite the newer options, the MEQ is still the most widely used questionnaire.

Actigraphy

Actigraphy is an easy non-invasive method of measuring the human rest-activity cycle. A small actimetry sensor is worn by the subject, often on the (non-dominant) wrist, to measure gross motor activity. It is based on the principle that there is increased movement during wake periods and reduced movement during sleep. The actimeter has to be worn continuously for at least 5 days to obtain reliable data on the subject's characteristics (Acebo et al., 1999). Recording activity is a standard as a marker of circadian rhythms in animal studies. In human studies, however, it has not (yet) reached this status.

Compared with the gold standard (polysomnography, PSG), actigraphy is a reliable and valid method for detecting sleep in a normal healthy adult population (Binnie et al., 1984; Van Someren and Nagtegaal, 2007) (Jean-Louis et al., 1996), but it becomes less reliable when sleep is more fragmented (Kushida et al., 2001). Actigraphy can be used as a complementary assessment to determine sleep patterns in patients suspected of certain sleep disorders, such as insomnia and restless legs or periodic limb movement disorder (Hauri and Wisbey, 1992; Sforza et al., 1999). Furthermore, it can be applied in subjects with suspected circadian rhythm sleep disorders, as actigraphy correlates well with sleep logs, PSG and markers of the circadian phase in patients with these disorders (Morgenthaler et al., 2007; Nagtegaal et al., 1998). Sleep disturbance resulting from, for instance, shift work or jet lag can also be detected by actigraphy (Ancoli-Israel et al., 2003). Finally, it can also be used when PSG is cumbersome, as actigraphy is non-invasive, less expensive, can be done at home, allows long-term continuous recording and can be used in populations in which PSG would be difficult (e.g., young children, patients with dementia (or) psychiatric disorders).

Although wrist activity appears to be a strong correlate of the entrained endogenous circadian phase, because of its susceptibility to masking and artefacts, actigraphy alone does not necessarily reflect the characteristics of the underlying circadian clock. Therefore, in the study of circadian rhythms, actimetry has certainly come to stay, but for now only as a good additional tool.

In our tertiary epilepsy centre we have set up a large prospective study, in which we measure circadian rhythms of patients with epilepsy who attend our centre for long-term EEG and video registration. The circadian rhythms of individual patients are correlated with recorded epileptic seizures during intake to elucidate the interaction between the biological clock and epilepsy. For this study, we use the DLMO (with night peak determination), CBT and questionnaires (MEQ and MCTQ) as measures of the circadian rhythm.

Summary

For more than a hundred years it has been known that in certain types of epilepsy, seizures tend to occur non-randomly. The reason for this has not been elucidated yet. One current hypothesis is that circadian rhythm plays a significant role. Results from animal studies suggest that seizures in experimental partial epilepsy are probably under the influence of the biological clock, but state-of-the-art studies on the influence of circadian rhythm on epilepsy in humans have not been conducted yet. To measure the circadian rhythm in humans, protocols are used to desynchronize the circadian rhythm from the sleep-wake cycle. Biological markers such as core body temperature, dim-light melatonin onset and cortisol level are used to determine the phase of the circadian rhythm and, if measured over more than one cycle, also the period of the endogenous circadian pacemaker. Certain parameters of sleep can be used to estimate circadian phase in humans; however, these are not used as frequently as the biological phase markers. Questionnaires can be employed to determine a person's chronotype, which is very informative about the circadian phase. Finally, actigraphy is not (yet) a reliable measure when used as the only measure, but it can be a very useful addition in studies of circadian rhythm.

Chapter Four

Temporal distribution of clinical seizures over the 24-h day: A retrospective observational study in a tertiary epilepsy clinic

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Epilepsia (2009) 2019-2026

Abstract

Very few studies have evaluated seizure occurrence in humans over the 24-h day; data from children are particularly scarce. Circadian patterns in seizure occurrence may be of importance in epilepsy research and may have important implications in diagnosis and therapy. We have analysed clinical seizures of 176 consecutive patients (76 children, 100 adults) who had continuous electroencephalography (EEG) and video monitoring lasting more than 22 hours. Several aspects of seizures were noted, including classification, time of day, origin and sleep stage. More than 800 seizures were recorded. Significantly more seizures were observed from 1100 to 1700h and from 2300 to 0500h significantly fewer seizures were seen. The daytime peak incidences were observed in seizures overall, complex partial seizures (in children and adults), seizures of extratemporal origin (in children) and seizures of temporal origin (in adults). Incidences significantly lower than expected were seen in the period 2300 to 0500h in seizures overall, complex partial seizures (in children and adults) and in tonic seizures (in children). In addition, significantly fewer seizures of temporal (in children and adults) and extratemporal origin (in children) were observed in this period. The results suggest that certain types of seizures have a strong tendency to occur in true diurnal patterns. These patterns are characterized by a peak during midday and a low in the early night.

Introduction

Epilepsy is a common neurologic disorder in which the unpredictability of seizure occurrence is an important cause of disability. However, in certain epileptic syndromes the occurrence of seizures is not totally unpredictable. Over a century ago, Gowers (1885) classified seizure occurrence as diurnal, nocturnal and diffuse. Later studies confirmed and extended his findings. It was observed that diurnal seizures cluster at certain times of the day, namely upon awakening and in the late afternoon and that nocturnal seizures tend to occur mainly at bedtime and in the hours before awakening (Langdon-Down & Brain, 1929; Griffiths & Fox, 1938).

Clear diurnal patterns of epileptic seizures have also been found in animal studies. For instance, generalized seizures after audiogenic stimulation are more frequent during darkness (Halberg & Howard, 1958) and animals kindle significantly more slowly during light than during darkness (Freeman, 1980). However, in later studies of rodents with limbic epilepsy it was observed that more spontaneous seizures occurred during light than during darkness (Cavalheiro et al., 1991; Bertram & Cornett, 1994; Quigg et al., 1998; Hellier & Dudek, 1999; Quigg et al., 2000; Gorter et al., 2001; Torshin & Vlasova, 2001). Seizure latency, in contrast to Freeman's results, was shorter during light periods. When clock times of seizures in animals were included, peaks for spontaneous seizures were found between 1200 and 1700h (Quigg et al., 1998).

The distribution of seizures during the human sleep-wake cycle has been studied extensively. Data on the temporal distribution of seizures over the 24-h day is, however, scarce. Up to now only eight studies are available, two of which are case reports (Halberg & Howard, 1958; Quigg & Straume, 2000). Two prospective studies, based on seizures diaries, did not lead to clear conclusions on whether seizures occur randomly or non-randomly (Milton et al., 1987; Tauboll et al., 1991). With newer diagnostic opportunities provided by continuous EEG monitoring, true epileptic seizures can be recorded more reliably. However, to our knowledge, these state of the art techniques have been used in only four studies (Binnie et al., 1984; Quigg et al., 1998; Pavlova et al., 2004; Durazzo et al., 2008). In one, individual epileptiform discharges in twelve 48-h EEG recordings were counted and non-random seizure distribution was found in approximately half, although with little consistency (Binnie et al., 1984). In another study, the pattern of partial seizure occurrence in adults was compared with seizures in an epileptic rat model (Quigg et al., 1998). It was found that in patients with mesial temporal lobe epilepsy (mesial TLE) seizures did not occur randomly, but in a cosinor daily distribution, comparable to the occurrence of seizures in rats. In a third study, focal seizures in adults were registered and clear patterns in seizure occurrence over the day in both TLE and extratemporal epilepsy (XTLE) groups were shown. One very recent study has used intracranial EEG monitoring to evaluate temporal distribution of seizures (Durazzo et al., 2008). The authors found non-uniform seizure distributions in seizures from the parietal, occipital, mesial temporal and neocortical temporal lobes, with peak occurrences on different moments of the 24-h day.

Although all these studies are informative, the first three have included small numbers of patients and seizures. Furthermore, in all four studies only focal or partial seizures in adult subjects were included and sleep and sleep stages were not addressed.

More knowledge about the potential circadian rhythm of seizure occurrence in epilepsy may contribute to our understanding of the pathophysiology of this disease. Moreover, it may

have significant clinical implications for the timing of therapy in epilepsy. Knowledge may also lead to improved quality of life of patients with epilepsy, by making seizures more predictable. We performed a large retrospective study in our tertiary epilepsy and sleep centre, including all children and adults with various types of epileptic seizures occurring during long term video and EEG monitoring over a 5-year period.

Methods

Subjects

In our tertiary epilepsy and sleep centre, patients are admitted for continuous EEG and video monitoring, varying in duration from 1 hour to 7 days. The recordings are performed for various reasons, mostly for classification of epilepsy or presurgical evaluation. Patients are monitored in a specially designed unit. During the day they stay in a living room. All patients have their own bedroom. The patients remain in his or her daily routine of meals and so on. Monitoring is performed according to the recommendations of Velis et al. (2007).

EEG and video monitoring is performed continuously, even over periods of many days. Furthermore, specially trained nurses are always present in the living room. When patients are in their bedrooms, they are constantly monitored by EEG and video with real-time human supervision.

From 2003 to 2007 EEGs of all consecutive patients with seizures during continuous EEG and video registration of 22 hours and longer were included. The subjects were divided into two groups according to age: children (1-15 years) and adults (16-65 years). Only clinico-electrographic seizures were included, that is, seizures with clinical symptoms (visible on video), as well as with simultaneous ictal activity on EEG (duration of at least 2 seconds). An example of epileptiform discharges is shown in Fig. 1.

EEG and video recording systems

EEG monitoring was performed by a 40-channel Lanotta long-term monitoring amplifier in combination with a Stellate Harmonie Epilepsy Monitoring System. Scalp recordings were performed using the 10-20 system with additional electrodes F9, F10, P9 and P10, according to the 10-10 system. Sub-mental electromyography (EMG), electro-oculography (EOG) and electrocardiography (ECG) electrodes were also used. Respiration was measured by abdominal piezo respiratory effort sensors. With these EEG and additional EMG, EOG, ECG electrodes and measurement of respiration not only seizures and other events could be monitored, but sleep could also be analysed conforming to the scoring rules of the American Academy of Sleep Medicine (AASM) 2007 (Silber et al., 2007). Video monitoring was performed simultaneously by closed circuit television and recorded from multiple remotely controlled Axis cameras. All EEG and video data were saved. After interpretation all material containing important clinical information was archived (Hewlett Packard).

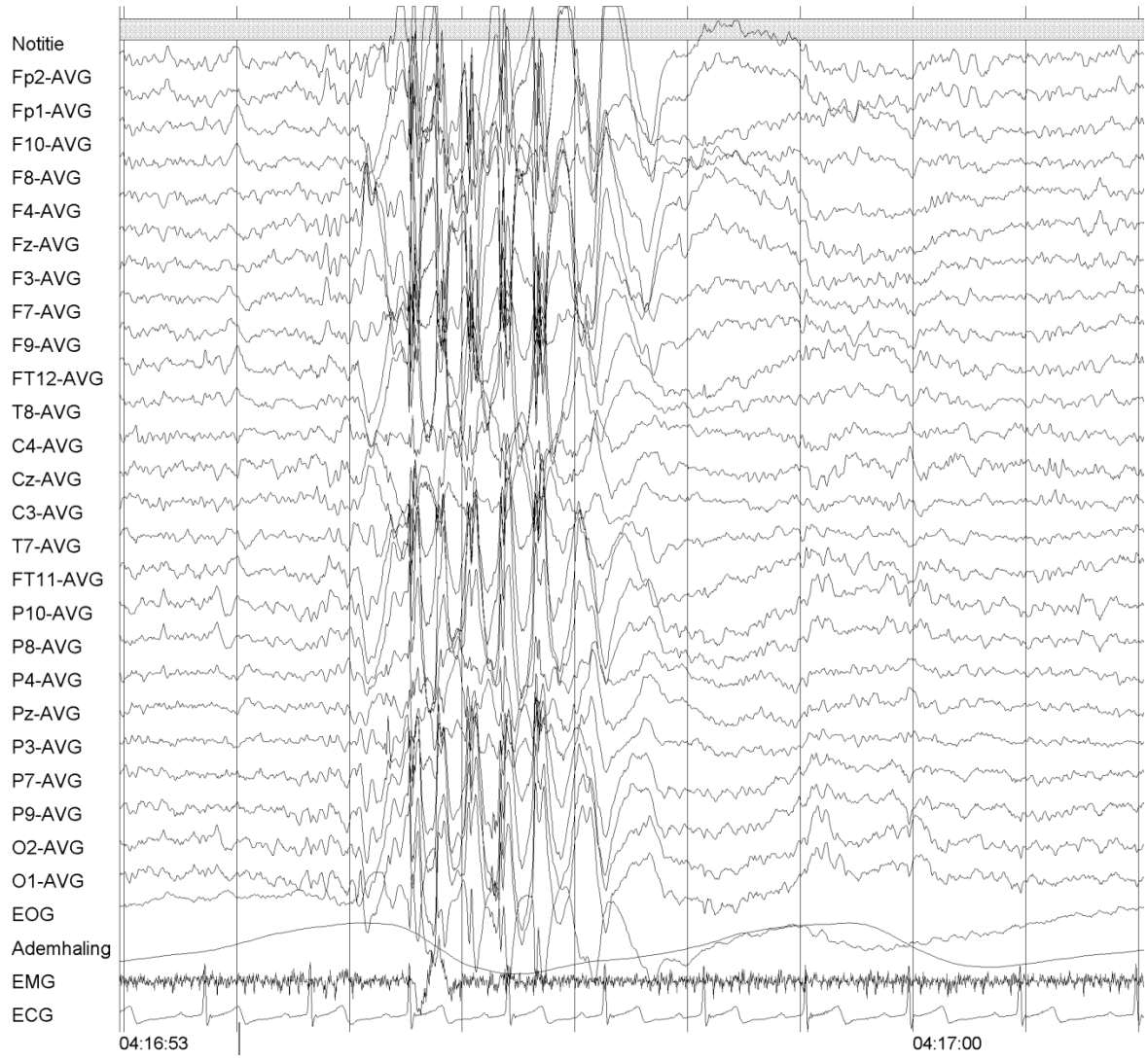

Figure 1. An example of a clinico-electrographic seizure with duration of slightly over 2 seconds. EOG, electro-oculography; EMG, electromyography; ECG, electrocardiography; Ademhaling (Dutch), respiration. Time between two vertical lines is 1 second.

Data analysis

We have evaluated entire recordings of EEG data and relevant video data at least twice. First by well-trained technicians of the clinical neurophysiology department and second by a board certified clinical neurophysiologist. In case of discrepancy, the interpretation of another clinical neurophysiologist was decisive. Retrospectively, time of occurrence of seizures was noted, including sleep stage when occurring during sleep. Seizures were classified according to the classification of the International League against Epilepsy of 1981 (ILAE Commission on Classification and Terminology, 1981). Origin of the seizures was determined by the onset of ictal activity in the EEG.

Because a full array of EEG, EMG, ECG and EOG electrodes was used, including measuring of respiration, sleep could be scored accurately according to the scoring rules of the AASM 2007, with one exception (Silber et al., 2007). Because the study regarded epilepsy, it was preferred to have low frontotemporal electrodes (FT11 and FT12) instead of ear electrodes (A1 and A2). As both locations are very close to each other, we decided to

use FT11 and FT12 electrodes for sleep scoring. For the sleep scoring itself the montage was reformatted to F3- FT12; F4- FT11; C3- FT12; C4- FT11; O1- FT12; O2- FT11. In the event of a seizure during sleep, sleep stage was determined from the epoch in which the seizure occurred. If this was not possible, for example, due to long duration of the seizure, the previous epoch was used to determine sleep stage.

The 24-h day was divided into four bins. With the approximate minimum of the core body temperature as a starting point, the time bins were 0500-1100h (I), 1100-1700h (II), 1700-2300h (III) and 2300-0500h (IV), consecutively.

The non-parametric binomial test was used to test whether numbers of seizures in the four time bins were significantly different. When assuming that seizures occur randomly, a percentage of 25% of the total number of seizures in each of the four bins is expected. The binomial test measures differences between the expected percentages and found percentages in the study. Significance was set at p-values of <0.001, <0.01 and <0.05. For statistical analysis SPSS v12.0.1 (SPSS, Chicago, IL, U.S.A.) was used.

Results

Included subjects

Over a period of 5 years, 176 patients (808 seizures) were included. The population consisted of 76 children [32 girls, 44 boys, mean age 8.3 years (range 1-15 years), mean duration of registration 26.3 h (range 22-96 h)] and 100 adults [54 women, 46 men, mean age 36.0 years (range 16-62 years), mean duration of registration 48.8 h (range 22-168 h)]. In the group of children, 396 seizures were observed and in the adults 412 seizures were documented. Thirteen children showed seizures of temporal origin (67 seizures) and 63 of extratemporal origin (329 seizures). In the group of adult patients, 65 patients had seizures of temporal origin (241 seizures) and 35 of extratemporal origin (171 seizures).

Temporal distribution of seizures over the 24-h day

Significantly more seizures were observed in the time period 1100-1700h. This was seen in total numbers of seizures and complex partial seizures in children and adults. In addition, significantly more seizures of extratemporal origin in children and seizures of temporal origin in adults were seen during this period. Significantly fewer seizures were seen during the night (2300-0500h): seizures overall and complex partial seizures in children and adults and in tonic seizures in children. Furthermore, a significant decrease of seizures of temporal origin in children and adults and of extratemporal origin in children was observed during this period.

Tables 1 and 2 present the numbers of seizures in children and adults, respectively, distributed according to classification and origin. Statistical analysis was possible for seizure types with the highest prevalence and for origins of seizures. A more detailed overview of temporal distribution of complex partial seizures is shown in Fig. 2 (time bins of 2 hours). Fig. 3 shows seizures of temporal and extratemporal origin in children (A) and adults (B) in more detail (also 2-h time bins). The figures show 24-h cosinor rhythmicity for complex partial seizures in both children and adults and also for seizures of extratemporal origin in children and seizures of temporal origin in adults.

Table 1. Temporal distribution in various types of seizures and origin in children, *n*=396

Time (Bin)	0500-1100h (I)	1100-1700h (II)	1700-2300h (III)	2300-0500h (IV)
Seizure type				
Simple partial ¶	3	13	7	0
Complex partial	61	84***	46	24***
Tonic partial	37*	30	25	15**
Clonic partial ¶	3	13	3	1
Sec. generalizing tonic/ clonic ¶	3	2	1	1
Myoclonic ¶	5	14	4	0
Origin				
Temporal	23‡	23‡	12	9*
Extratemporal	89	134***	74	32***
(Frontal)	(49)	(79) ***	(39)	(4)***
Total	112	156***	86	41***

* p<0.05; ** p<0.01; *** p<0.001; ‡ p=0.056 (binomial test); ¶ The small numbers of seizures in these categories limit statistical analysis; Sec, secondary.

Table 2. Temporal distribution in various types of seizures and origin in adults, *n*=412

Time (Bin)	0500-1100h (I)	1100-1700h (II)	1700-2300h (III)	2300-0500h (IV)
Seizure type				
Simple partial ¶	16	17	20	13
Complex partial	63	93***	57	52*
Tonic partial ¶	10	11	11	4
Clonic partial ¶	3	1	0	1
Sec. generalizing tonic/ clonic ¶	4	13	6	0
Myoclonic ¶	7	3	7	0
Origin				
Temporal	60	90***	62	29***
Extratemporal	43	48	39	41
(Frontal)	(33)	(26)	(20)‡	(31)
Total	103	138***	101	70***

* p<0.05; ** p<0.01; *** p<0.001; ‡ p=0.058 (binomial test); ¶ The small numbers of seizures in these categories limit statistical analysis; Sec, secondary.

Table 3. Seizures during sleep in children and adults, divided according to origin

Sleep stage	NREM I/II	SWS	REM	Total in sleep (percentage of n)
Seizures in children (n=396)	*39*	*13*	*11*	*63 (15.9%)*
Temporal origin (n=67)	11	3	10	24
Extratemporal origin (n=329)	28	10	1	39
Seizures in adults (n=412)	*57*	*21*	*5*	*83 (20.1%)*
Temporal origin (n=241)	23	7	1	31
Extratemporal origin (n=171)	34	14	4	52
Total (n=808)	96	34	16	146 (18.1%)

NREM, non-rapid eye movement; REM, rapid eye movement; SWS, slow wave sleep

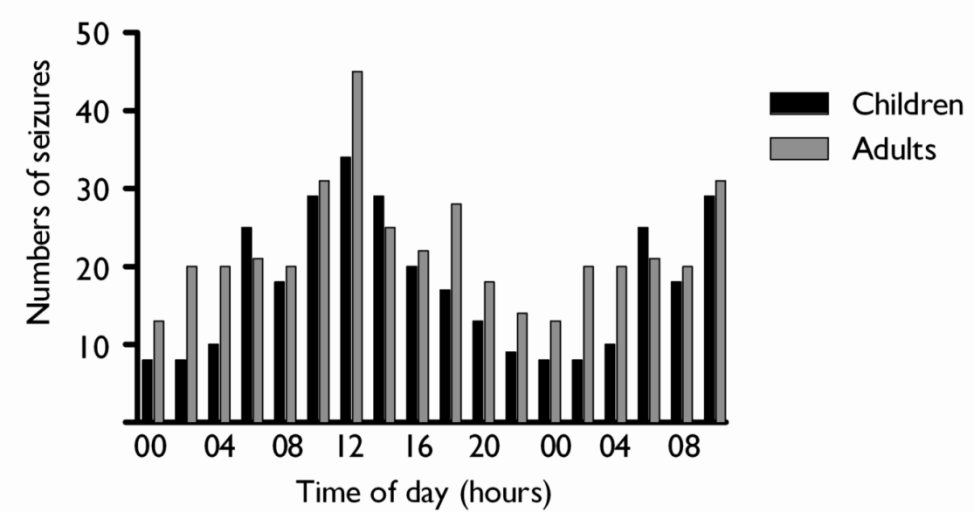

Figure 2. Bar histogram showing temporal distribution of complex partial seizures in children and adults in more detail (numbers of seizures are n = 215 and n = 265 respectively). Each bar represents numbers of seizures per 2 hours (e.g., '00' is from 00-02 h). Data of 36 hours is given in order to show the 24-h cosinor rhythmicity.

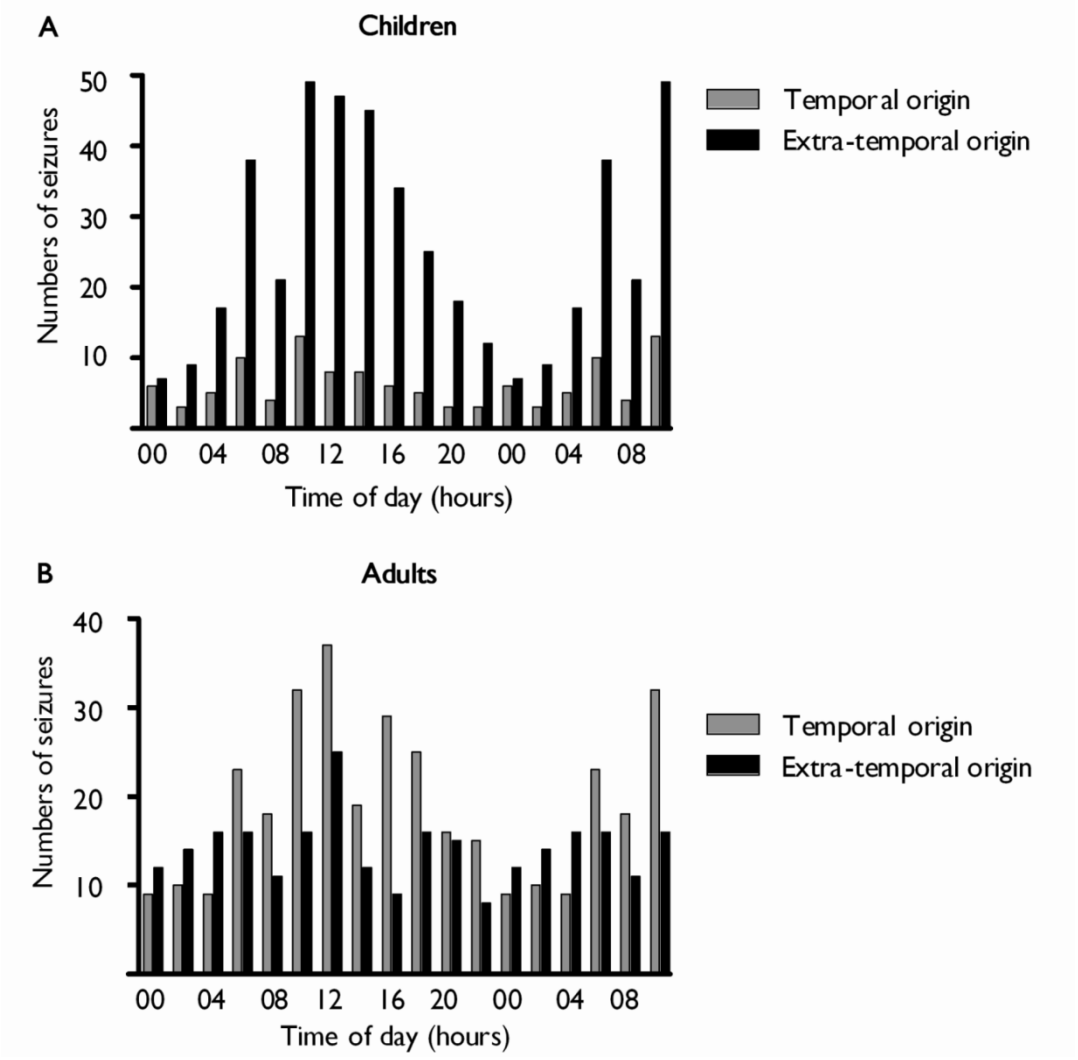

Figure 3. Bar histogram showing temporal distribution of seizures of temporal and extratemporal origin in more detail in children (A) and adults (B). Each bar represents numbers of seizures per 2 hours (e.g., '00' is from 00-02 h). Data of 36 hours is given in order to show the 24-h cosinor rhythmicity.

Sleep stages

Approximately 18% of all seizures were observed during sleep. Most of these seizures were observed during non-rapid eye movement (NREM) sleep I/ II, fewer seizures during deep sleep (slow wave sleep, SWS) and even fewer during rapid eye movement (REM) sleep (Table 3).

Discussion

Overall, significantly more clinical seizures were observed in the period of 1100-1700h and during the night (2300-0500h) significantly fewer seizures occurred. This was observed in several seizure types as well as seizures from different parts of the brain.

Twenty-four hour patterns in seizure occurrence have already been observed in animal studies (Quigg et al., 1998, 2000). In humans, however, very few studies have addressed this subject. The temporal distribution of seizures that was found in our study is comparable to the distribution Halberg and Howard found in two patients who were followed for many years (Halberg & Howard, 1958). In another case study, Quigg and Straume found a similar distribution in an adult with temporal seizures based on a seizure diary maintained for 5 years (Quigg & Straume, 2000). In three other reports, seizure occurrence was not specified to clock times, but when all three studies are taken together, non-random seizure patterns were found in 53% of the 66 subjects (Binnie et al., 1984; Milton et al., 1987; Tauboll et al., 1991). Unfortunately, in two of these studies, only seizure diaries were used (Milton et al., 1987; Tauboll et al., 1991). This is not as reliable as EEG monitoring, as seizures during the night can be missed and psychogenic seizures can be confused with epileptic seizures. A more recent study did use EEG monitoring to explore the temporal distribution of partial seizures in adults, which was compared to an animal model (Quigg et al., 1998). This elaborate study compared the patterns of partial seizures in 96 patients with mesial TLE (MTLE), lesional TLE (LTLE) and XTLE. It was found that partial seizures in LTLE and XTLE strike randomly; however, a distinct pattern was found in partial seizures in MTLE, with a peak incidence at 1500h. This pattern is comparable to our results. We could not subdivide our data, as a final diagnosis cannot be made in all of our patients according to the present ILAE-criteria, because imaging of the brain is sometimes not available. However, even without this subdivision, a clear pattern emerged. Applying the subdivision used by Quigg to our data could lead to an even more pronounced pattern.

In another EEG monitoring study, Pavlova et al. described 26 patients (90 seizures) and found a significant peak in seizure occurrence in the time bin of 1500-1900h in TLE patients and a peak in the bin of 1900-2300h in XTLE patients (Pavlova et al., 2004). Comparing our results to these studies, however, is difficult as different criteria were used for including patients in the TLE groups. We have subdivided seizures strictly according to the onset of ictal activity on the EEG, whereas other authors included patients on clinical grounds, EEG monitoring and imaging of the brain.

Finally, Durazzo et al. have studied temporal distribution of seizures using intracranial EEG monitoring (Durazzo et al., 2008). In this study 669 seizures of 131 adult patients were analysed. They found that seizure distribution was dependent on brain region. Non-uniform seizure distributions were observed in seizures from the parietal, occipital, frontal, mesial temporal and neocortical temporal lobes. When comparing their results from temporal seizures to ours, we find a resemblance in the afternoon time bins in which seizures of

temporal origin peak. The second peak prevalence found in their study (0700-1000h) does not correspond to our results, however.

Our findings indicate that frontal seizures in adults occurred more often during the night than during the day; however, this difference was only borderline significant (p=0.058 in bin III, 2300-0500h). When comparing to known literature, we would expect significantly more frontal seizures during the night (Panayiotopoulos, 2007). In children, more frontal seizures were seen during the day.

Our results confirm previous findings of more seizures in NREM sleep, fewer seizures during deep sleep and very few seizures in REM sleep (Herman et al., 2001;Herman & Walczak, 2002). The observed percentage of seizures during sleep in adults is also consistent with previous work (Bazil & Walczak, 1997). The observed percentage in children, however, differs greatly from the 41% observed in a study by Herman et al. (Herman et al., 1999). Other percentages of seizures during sleep in children are not known; therefore, further comparison is unfortunately not possible. The reason for the difference between the found percentages is not clear.

The strengths of our study are that this is the first that differentiates more precisely the occurrence of seizures within the 24-h day in children and as well as adults in various types of seizures, including sleep and sleep stages. Furthermore, we have used EEG and video monitoring, which guarantees very accurate seizures counts. In addition, because entire recordings of EEG have been reviewed at least twice by well-trained neurophysiologic technicians and a board certified clinical neurophysiologist, this minimizes the chance of missing seizures or erroneous interpretation.

Our study also has several limitations. First, our database from a tertiary epilepsy clinic does not represent the average population of epilepsy patients. Complex partial seizures contribute disproportionally and primary generalized seizures are not represented at all; all generalized seizures in our analysis are secondary generalized seizures. Likewise, in this population, the distribution of temporal versus extratemporal seizures in children and adults is different from expected in the average population. This is probably also due to the fact that these are patients from a tertiary clinic. Furthermore, numbers of some other types of seizures are too small to produce significant patterns. For most of these types, group trends can be seen, however. Despite these limits, our study produces valuable information about the temporal distribution of different types of seizures.

Secondly, superficial EEG monitoring was used. As a consequence, some epileptiform activity in the brain may not be captured by the electrodes. As we only included seizures that showed ictal activity in the EEG, clinically suspect seizures without ictal activity in the EEG were excluded, for example, simple partial seizures. We next plan to study temporal distribution of seizures in patients with intracranial recording in order to circumvent this limitation and to compare our data with the results of the study by Durazzo et al. (Durazzo et al., 2008).

Thirdly, as we have only included seizures with clinical symptoms, as well as with simultaneous ictal activity in the EEG, there is a chance that we have excluded seizures with very subtle clinic. Especially during the night, when the patient is lying in bed, covered with bed linen, very subtle clinical seizures can be missed.

Finally, it is not known to what extent seizures and seizure timing are influenced by endogenous and exogenous factors. For instance, daily activities such as physical exercise and mealtimes may influence the timing of seizures. We do find peaks in seizure occurrence

from 1200-1400h (lunch time) in complex partial seizures as well as in temporal seizures in adults. In our centre there are three meal times a day; two bread meals (0800h and 1200h) and supper at 1730h. However, apart from a small insignificant peak in complex partial seizures in adults (1800-2000h), no effects can be seen during and directly after breakfast and supper. In addition, within the 1200-1400h period, seizures are equally distributed. No significant differences are seen in seizure occurrence during and after lunch time. These findings suggest no direct influences from the meal itself. What causes this intriguing peak in seizure occurrence from 1200-1400h is not clear and remains to be clarified. Furthermore, the extent of effects of antiepileptic drugs (AEDs) on the distribution of seizures remains to be elucidated. Because patients cannot be registered under constant conditions (Duffy & Dijk, 2002), the precise influence of these factors on seizures and patterns of seizure occurrence cannot be predicted. This will be a subject of further study in our laboratory.

There may be important consequences for daily practice and research if seizures indeed occur in patterns. Insight into seizure patterns can help in understanding the pathophysiology of epilepsy. Furthermore, such patterns imply that diagnostic options (such as EEG monitoring) have to be adjusted to the expected type and/or origin of seizures. For instance, short-term EEGs to capture complex partial seizures can preferably be scheduled at midday or in the afternoon, as our results strongly support that these seizures tend to occur more frequently in that period. Finally, adjustment of therapy to individual circadian patterns may improve effects of AEDs.

In conclusion, our study makes clear that certain types of seizures do not occur randomly, but take place in daily patterns. Although the recognition of patterns in seizure occurrence is an important first step, more research is needed to further explore these daily patterns and to identify underlying mechanisms that lead to these temporal rhythms of seizures.

Chapter Five

Diurnal rhythms in seizures detected by intracranial electrocorticographic monitoring: An observational study

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Epilepsy & Behavior (2009) 617-621

Abstract

Few studies have evaluated human seizure occurrence over the 24-hour day and only one group has employed intracranial electrocorticography monitoring to record seizures. Circadian patterns in seizures may have important implications in diagnosis and therapy and provide opportunities in research. We have analysed spontaneous seizures in 33 consecutive patients with long-term intracranial EEG and video monitoring. Several aspects of seizures were noted, including time of day, origin, type and behavioural state (sleeping or awake). We recorded 450 seizures that showed an uneven distribution over the day, depending on lobe of origin: temporal lobe seizures occurred preferentially between 1100 and 1700h, frontal seizures between 2300 and 0500h and parietal seizures between 1700 and 2300h. In the awake state, larger proportions of clinical seizures were seen from 0500 to 1100h and from 1700 to 2300h. During sleep, larger proportions occurred from 1100 to 1700h and from 2300 to 0500h. Our results suggest that seizures from different brain regions have a strong tendency to occur in different diurnal patterns.

Introduction

The unpredictability of epileptic seizures is an important factor in the disabling character of the disease. It has been shown that not all seizures occur randomly (Binnie et al., 1984; Griffiths and Fox, 1938; Hofstra et al., 2009a; Milton et al., 1987; Tauboll et al., 1991). Animal studies have provided some answers, as clear diurnal patterns of seizures have been observed in various epilepsy models. For instance, in studies of rodents with limbic epilepsy, it was observed that seizure latency was shorter and more spontaneous seizures occurred during exposure to light than during darkness (Bertram and Cornett, 1994; Cavalheiro et al., 1991; Gorter et al., 2001; Hellier and Dudek, 1999; Quigg et al., 1998b; Quigg et al., 2000; Torshin and Vlasova, 2001).

In humans, patterns of seizure occurrence have been studied, but most authors have assessed only the random or non-random character of these patterns (Binnie et al., 1984; Milton et al., 1987; Tauboll et al., 1991). Data on precise temporal distribution of seizures, however, are particularly scarce. Four studies have provided details on the temporal distribution of seizures using long-term EEG monitoring, three of these four studies used scalp EEG monitoring (Hofstra et al., 2009a; Pavlova et al., 2004; Quigg et al., 1998b). In one study, the pattern of partial seizure occurrence in adults was compared with that of seizures in an epileptic rat model (Quigg et al., 1998b). It was found that in patients with mesial temporal lobe epilepsy (MTLE), seizures did not occur randomly, but in a sinusoidal daily distribution, comparable to the occurrence of seizures in rats. In another study, clear patterns in both temporal lobe seizures and extratemporal seizures were demonstrated (Pavlova et al., 2004). In a recent study, we evaluated the temporal distribution of different types of clinical seizures also using scalp EEG. We observed significantly more seizures from 1100 to 1700h and significantly fewer seizures from 2300 to 0500h (Hofstra et al., 2009a).

These three studies have provided valuable information; however, they all used scalp EEG monitoring to record seizures. Scalp EEG may not detect all seizures, especially more localized or subtle seizures and seizures arising from deep structures that cannot be detected on the scalp. In addition, muscle artefacts may obscure a fast seizure EEG rhythm. At present, intracranial electrocorticography (ECoG) monitoring (IEM) is the gold standard for recording seizures and delineating epileptic foci without these limitations. To our knowledge, only one recent study used IEM to evaluate the temporal distribution of seizures (Durazzo et al., 2008). These authors found seizures from the parietal, occipital, mesial temporal and neocortical temporal lobes to be distributed non-uniformly, with peak occurrences at different times over the 24-h day. In that study, however, only seizures in adults were included and the behavioural state during seizures (sleep or awake) was not addressed.

To understand the pathophysiology of epilepsy, further details on circadian patterns in seizure occurrence need to be ascertained, preferably using IEM for accurate seizure detection. This knowledge may have significant clinical implications for diagnostic procedures timing of therapy in epilepsy. Therefore, we performed a retrospective analysis of all children and adults with various types of seizures who recently underwent long-term intracranial ECoG monitoring in the nationwide epilepsy surgery program in The Netherlands.

Methods

Intracranial ECoG monitoring and video recording systems

Long-term IEM was performed in the Intensive Epilepsy Monitoring Unit at the University Medical Centre in Utrecht. Subdural strips and grids were used for IEM. Strips were eight-electrode arrays made of 5-mm-diameter platinum disks spaced 10 mm between centres. Grids consisted of a multiple of eight 5-mm-diameter platinum disks (maximum of 64) in a rectangular array with 10-mm centre-to-centre distance (Ad-Tech, Racine, WI, USA). In one patient, placement of subdural strips through burr holes was sufficient. In all other patients, subdural strips and grids were placed after craniotomy. Grids were positioned depending on the suspected foci in the particular patient. Additional scalp EEG electrodes were placed according to the 10-20 or 10-10 system. ECoG was recorded with Telefactor equipment (Philadelphia, PA, USA; SF 512 Hz, 16 bits) from 1998 until 2005 and with Micromed equipment (Treviso, Italy; SF 512-2048 Hz, 16-22 bits) from 2005 onward. Furthermore, simultaneous polygraphy (electromyography, electro-oculography, etc.) and electrocardiography were performed. Respiration was monitored with abdominal piezo respiratory effort sensors. Patients were observed continuously through video monitoring. ECoG and video data were saved and archived after interpretation.

Before IEM, antiepileptic drugs (AEDs) were (partially) tapered, tailored to the individual patient. Other medication remained unchanged. Because of the monitoring equipment, patients had to remain in bed during the entire registration. Patients were allowed to sleep at their normal bedtimes and naps during the day were allowed, although they were sometimes disturbed by tests, physician's rounds, mealtimes and visiting hours. Monitoring was performed as long as clinically necessary. When the decision was made to resect the epileptic focus, the intracranial grids and strips were removed in the same procedure.

Inclusion

ECoG recordings of 33 consecutive patients with spontaneous seizures during IEM performed from 1999 to 2008 were included. A seizure was defined as an electroencephalographic epileptic event lasting at least 5 seconds. On the basis of remarks made by the patients and staff and close analysis of the videos, the seizures were assessed as subclinical or as having a clinical correlate. The latter included those limited to an aura.

A seizure cluster was considered one seizure, with the starting point of the first seizure as the start point. Seizures occurring after provocation, for example, hyperventilation or sleep deprivation, were not included. Patients with more than 15 seizures per time bin (see below) on a given day were excluded, as this could lead to erroneous peaks in seizure occurrence. The ECoG data together with the clinical aspects were decisive in determining the origin of each seizure.

Data analysis

Entire ECoG recordings were evaluated in Utrecht by well-trained technicians of the Clinical Neurophysiology Department and by board-certified clinical neurophysiologists (F.S.S.L., Dr. C.Y. Ferrier). Recent 1.5- or 3-T MRI, PET and sometimes ictal SPECT scans were available. These data together with other relevant information were reviewed before IEM by the Dutch National Task Force on Epilepsy Surgery, which includes clinical neurophysiologists, neurosurgeons and psychologists from all epilepsy centres in The

Netherlands. From the IEM data, time of occurrence of seizures was noted. Furthermore, occurrence during sleep or while awake, type of seizure (auras, subclinical or clinical) and lobe of origin of the seizures were determined.

To study the temporal distribution of seizures, the 24-h day was divided into four 6-h bins. With the expected nadir of core body temperature (approximately 0500h) as starting point, the time bins covered the periods 0500-1100h (I), 1100-1700h (II), 1700-2300h (III) and 2300-0500h (IV) hours. The non-parametric binomial test was used to test whether numbers of seizures in the four time bins were significantly different. Under the assumption that seizures occur randomly, the percentage of the total number of seizures in each of the four bins is expected to be 25%. The binomial test measures differences between the expected percentages (25% per time bin) and the percentages calculated in the study. Significance was set at a *p*-level of 0.05. For statistical analysis, SPSS Version 12.0.1 was used.

Results

Subjects

From 1999 to 2008, the seizures of 33 patients were analysed. The population consisted of 26 adults (9 women: mean age 29.7 years, range 17-45; mean duration of IEM 4.6 days, range 2-8) and 7 children (4 girls: mean age 11.6 years, range 5-15; mean duration of IEM 4.0 days, range 2-6).

Imaging of the brain with MRI revealed dysplasia in 13 patients, a tumour in 5, mesiotemporal sclerosis (MTS) in 3 and multiple causes (cavernomas and MTS) in one. In another five patients various lesions were found (a cyst in two patients, a post abscess tissue scar, posttraumatic and postsurgical defects); six patients had normal MRI scans. Eight other patients were excluded, because four had more than 15 seizures in one time bin, three had no spontaneous seizures and one had only daytime registrations.

Distribution of seizures over the day

A total of 450 spontaneous seizures were available for study. The origin was mesial temporal in 85 seizures (6 patients, 5 adults), neocortical temporal in 72 seizures (8 patients, 7 adults), frontal in 190 seizures (14 patients, 10 adults), parietal in 99 seizures (4 patients, 3 adults) and occipital in four seizures of one adult. Although differences between the temporal distribution of seizures in children and adults were not expected, seizures in adults (n=312) were also analysed separately in order for the results to be compared with results of other studies that analysed seizure occurrence only in adults.

Because of scarce data on seizures of occipital origin, these were excluded from analysis.

In seizures of mesial temporal origin, there was a significant peak in the number of seizures from 1100 to 1700h (p=0.002) and fewer seizures than average from 0500 to 1100h (p=0.005). A similar peak (1100-1700h) was observed for seizures of neocortical temporal origin (p=0.07). Frontal seizures occurred significantly more often during the night time (2300-0500h, p=0.049). Seizures of parietal origin peaked from 1700 to 2300h (p=0.008) and were less prevalent from 1100 to 1700h (p=0.024).

When seizures of adults were analysed separately, the results were as follows. The distribution of seizures of mesial temporal origin was similar: more seizures from 1100 to 1700h (p=0.002) and fewer from 0500 to 1100h (p=0.001). Fewer seizures from the frontal

lobe were seen from 1700 to 2300h (p=0.036); this was also observed for seizures from the parietal lobe (1700-2300h, p<0.001). When seizure occurrence in children was evaluated, only frontal seizures (85 seizures in four children) could be analysed reliably. It was observed that in these children fewer seizures occurred in the morning (0500-1100h) than during the rest of the day (p=0.04).

Table 1 lists the numbers of seizures per time bin, distributed according to origin. Fig. 1A illustrates the distributions of seizures of the four probable origins over time in the total population; Fig. 1B does the same for seizures in adults.

To determine whether time of day influences the clinical expression of seizures, we analysed the proportions of subclinical and clinical seizures per time bin. There were no significant differences between the proportions of subclinical and clinical seizures over the 24-h day (χ2 test, p=0.42).

As could be expected, most of the seizures of all origins while the patient was awake occurred during the day. Likewise, most of the seizures during sleep occurred during the night. On comparison of the clinical severity of seizures per time bin, larger proportions of seizures that occurred in the awake state were seen in the morning (0500-1100h) and evening (1700-2300h). Among seizures that occurred during sleep, larger proportions occurred during midday (1100-1700h) and midnight (2300-0500h) (Table 2). The distribution of seizures in the awake state or during sleep over the 24-h day with respect to origin is illustrated in Fig. 2. The small numbers of seizures in most groups limited statistical analysis of these data.

Table 1. Temporal distribution in seizures of various origins in children and adults, n= 450

Time (Bin)	0500-1100h (I)	1100-1700h (II)	1700-2300h (III)	2300-0500h (IV)
Origin				
Mesial Temporal	11**	34**	20	20
Neocortical Temporal	15	24[§]	15	18
Frontal	41	50	41	58*
Parietal	27	16*	36**	20
Occipital	1	3	0	0
Sum	95	127	112	116

* p<0.05; ** p<0.01; [§] p=0.07 (binomial test)

Table 2. Clinical severity of seizures during wake *(n=222)* and sleep *(n=228)* in children and adults

Time	0500-1100h	1100-1700h	1700-2300h	2300-0500h	All day
Wake (n)	*41*	*94*	*67*	*20*	*222*
Clinical (%)	90.2	64.9	91	75	78.4
Subclinical (%)	7.3	25.5	4.5	20	15.3
Aura (%)	2.4	9.6	4.5	5	6.3
Sum (%)	100	100	100	100	100
Sleep (n)	*54*	*33*	*45*	*96*	*228*
Clinical (%)	68.5	78.8	67	77.1	73.2
Subclinical (%)	31.5	21.2	33	22.9	26.8
Sum (%)	100	100	100	100	100

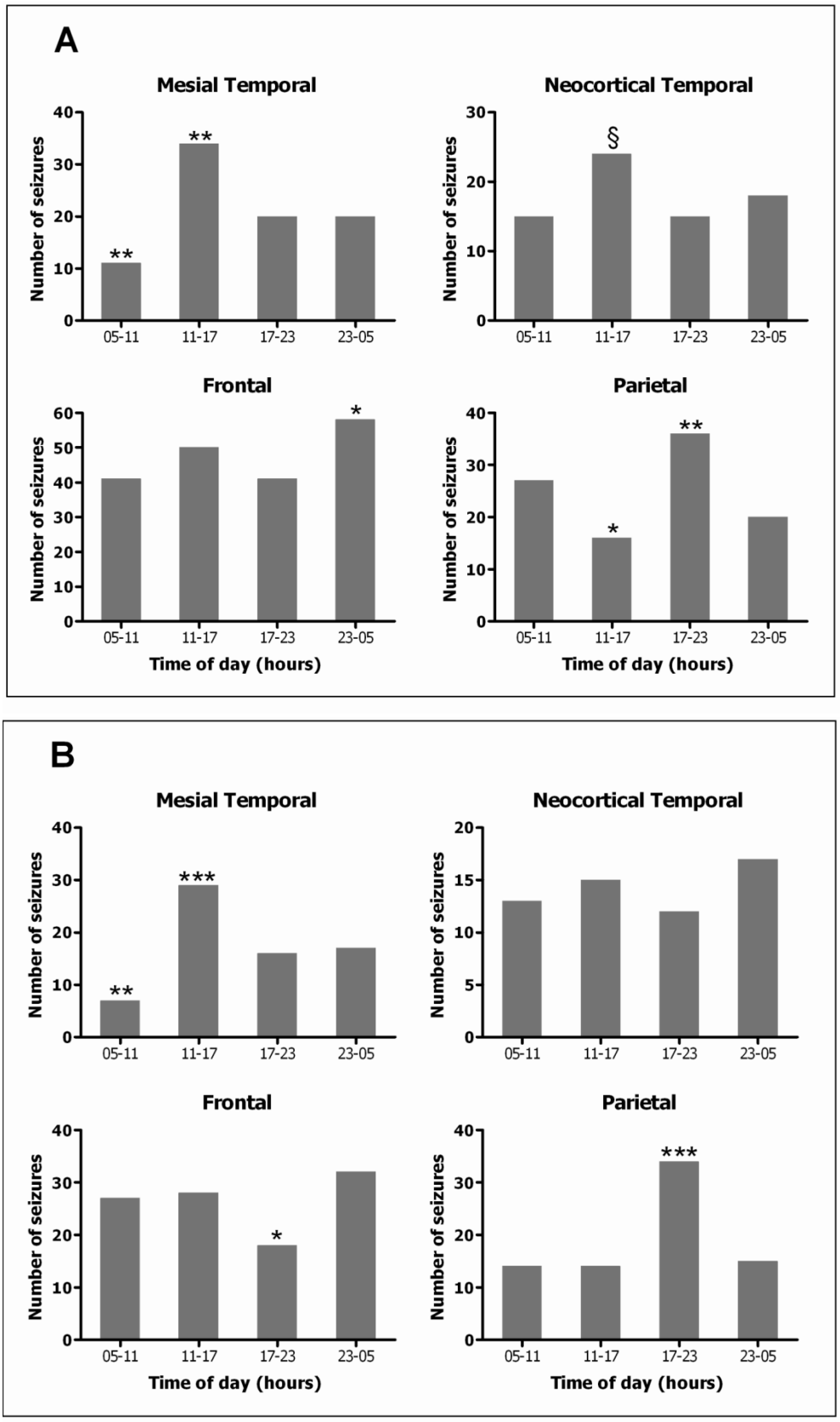

Figure 1. Bar histogram showing temporal distribution of seizures from different regions of the brain in children and adults (A, n = 446) and adults only (B, n = 308). Each bar represents number of seizures per 6-h time bin (*p<0.05, ** p<0.01, § p=0.07, *** p<0.001).

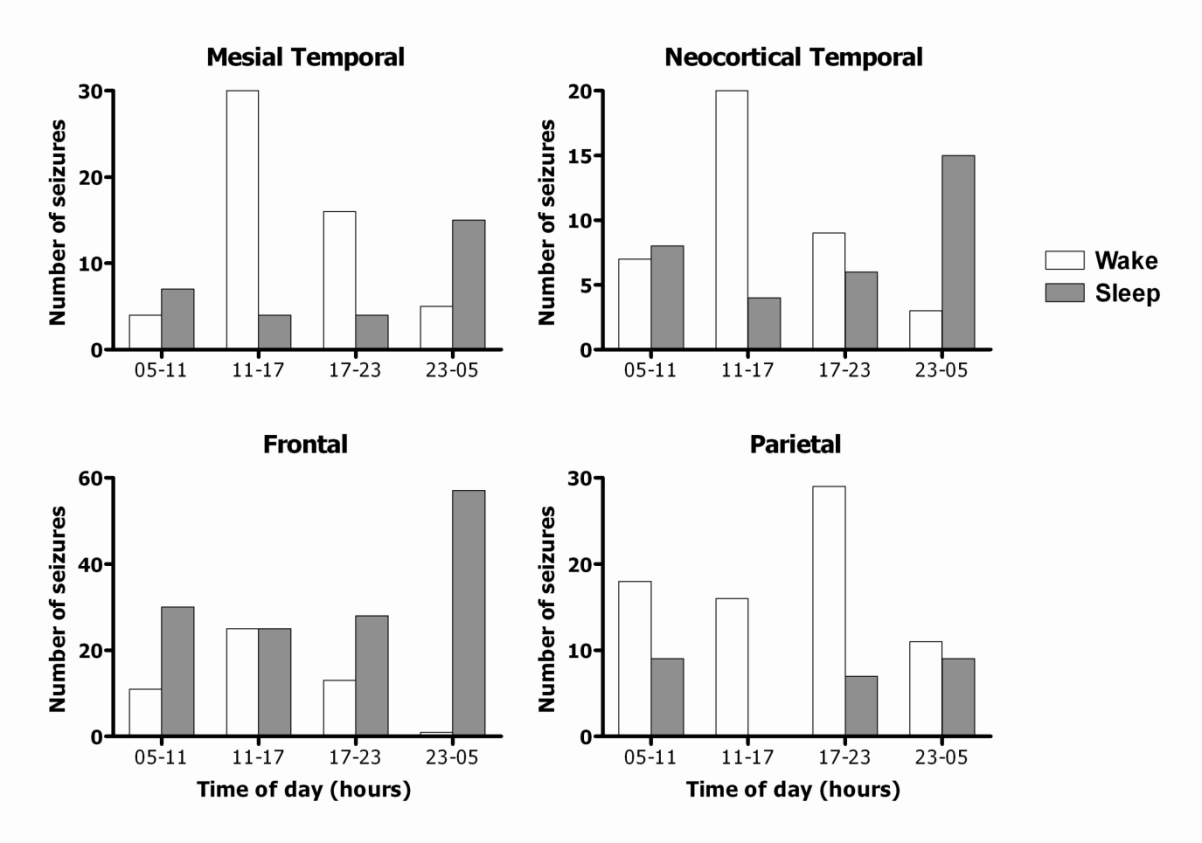

Figure 2. Bar histogram showing temporal distribution of seizures while awake or during sleep.

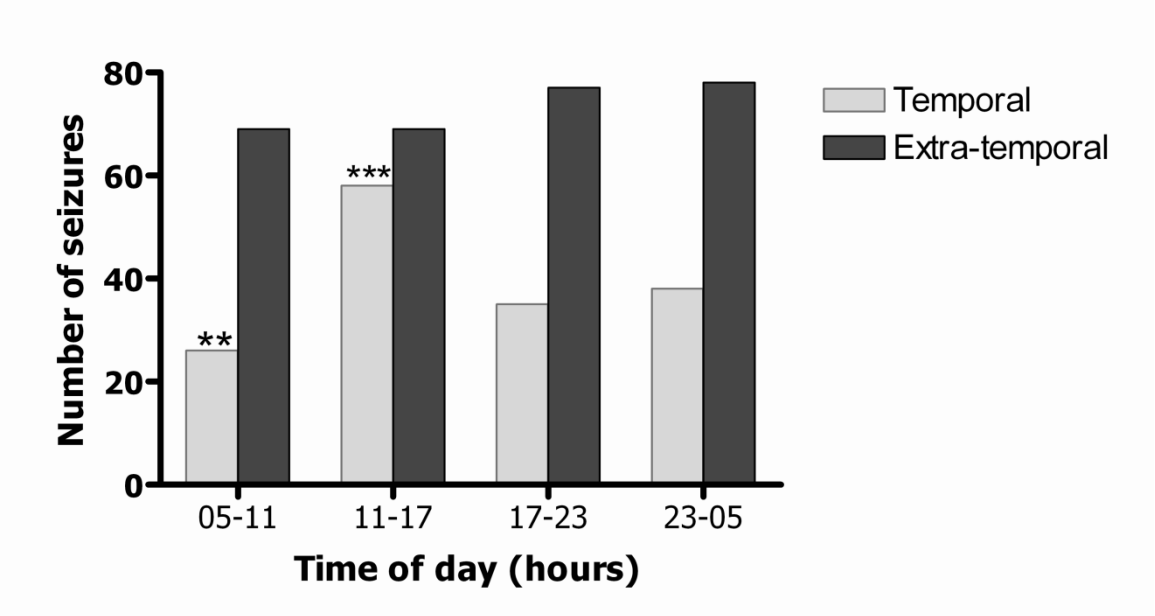

Figure 3. Bar histogram showing temporal distribution of seizures from the temporal lobe and the extratemporal lobes (**p<0.01, ***p=0.001).

Discussion

In our patients undergoing IEM, seizures of mesial temporal, neocortical temporal, frontal and parietal origin occurred in a non-random distribution over the 24-h day. Depending on the lobe of origin, the peak prevalence during the 24-h day differed.

Temporal distribution of seizures in humans has been studied previously, although knowledge is scarce. In three studies in which seizure diaries were used, seizure occurrence was not specified with respect to clock time. When all three studies are taken together, non-random seizure patterns were observed in 53% of the 66 subjects (Binnie et al., 1984; Milton et al., 1987; Tauboll et al., 1991). Unfortunately, seizure diaries are not as reliable as EEG monitoring, as nocturnal seizures can be missed, patients can have amnesia for their seizures and psychogenic seizures can be confused with epileptic seizures.

Only three studies have used scalp EEG. The first study regarded patterns of seizures in 96 adult patients with MTLE, lesional TLE and extratemporal lobe epilepsy (Quigg et al., 1998b). Seizures in patients with lesional TLE and extratemporal lobe epilepsy were found to be distributed randomly; however, seizures in patients with MTLE peaked at 1500h. In another study describing 26 patients (90 seizures), the seizures of those with TLE peaked from 1500 to 1900h and seizures in patients with extratemporal lobe epilepsy peaked from 1900 to 2300h (Pavlova et al., 2004). In this extraTLE group, five patients with a posterior (parieto-occipital) focus showed a peak in proportion of seizures between 1900 and 2300h. Comparison of these results with those of the adults in our IEM study revealed similarity in the prevalence late in the morning and early afternoon for seizures originating from the mesial temporal lobe; however, the peaks in our study were somewhat earlier. When our results for seizures of frontal, parietal and occipital origin are combined, an extratemporal pattern can be determined; this did not result in a significant pattern (see Fig. 3). Our results therefore support those of Quigg et al., rather than those of Pavlova et al., although the latter group's results regarding parieto-occipital seizures do tend to echo our results for seizures of parietal origin.

Recently, we analysed clinical seizures of 100 adults and 76 children who underwent continuous scalp EEG and video monitoring (mean duration 48.8 hours) (Hofstra et al., 2009a). For 808 seizures, time of day, classification, origin and sleep stage were noted. Significantly more seizures were observed from 1100 to 1700h and significantly fewer seizures from 2300 to 0500h.

Our current results may be different from the results of these EEG-based studies for several reasons. First, IEM is more accurate in recording seizures, as the seizures are not obscured by muscle artefacts and areas that are not accessible by scalp EEG can be recorded. Also, patient populations differ, as IEM is performed only in presurgical evaluation. These are patients with particularly severe focal epilepsy that is often difficult to localize by non-invasive techniques. Finally, the inclusion criteria in our scalp EEG study differ from those in this study: in our previous study only clinical seizures were included, whereas in this study the definition of a seizure was based mainly on the EEG.

Only one other group has studied the temporal distribution of seizures using IEM. Durazzo et al. analysed 669 seizures of 131 adult patients (Durazzo et al., 2008). Different non-uniform seizure distributions were observed in seizures of parietal, occipital, frontal, mesial temporal and neocortical temporal lobe origin. Several similarities can be observed between their results and our results in adults. The afternoon time bins in which seizures of

mesial temporal origin peak overlap in the two studies. However, the second peak in Durazzo and colleague's study (0700-1000h) does not correspond to our results, as in our patients significantly fewer seizures were documented. We observed fewer frontal seizures from 1700 to 2300h, which corresponds in part to the low (1600-1900h) in the study by Durazzo et al. Finally, we observed more parietal seizures from 1700 to 2300h. This distribution differs substantially from the Gaussian-like distribution with a low in the afternoon and early evening found by Durazzo et al., though our results are similar to those in patients with a parieto-occipital focus in the scalp EEG study of Pavlova et al., as noted above (Pavlova et al., 2004).

With respect to the methodology of these two studies, a few differences can be noted. First, Durazzo et al. were able to include more patients and seizures, but they used eight randomly chosen seizures per patient, while we included all spontaneous seizures of our patients, handling strict exclusion criteria. Also, they chose to use 3-h time blocks instead of our 6-h time bins. These distinctions may explain the differences in results.

Several strengths of our study can be mentioned. First, the gold standard IEM was used to record seizures. All ECoG recordings were reviewed at least twice and all subjects were discussed at length by the Dutch National Task Force on Epilepsy Surgery, which determined the epileptic focus precisely on the basis of a concordance of IEM, imaging, patient's history and clinical observations. Also, our IEM study is the first to include children as well as adults. Furthermore, we are the first to review seizures with IEM during both the awake state and sleep. Seizures from all parts of the brain (apart from the occipital lobe) were taken into account and analysed separately. Finally, by excluding patients with more than 15 seizures per time bin, counting a seizure cluster as one seizure and excluding provoked seizures, we prevented erroneous peaks in seizure occurrence.

A number of limitations remain. First, our database of patients undergoing IEM does not represent the average population of patients with epilepsy. Second, the extent of influence of endogenous and exogenous factors on seizures and seizure timing is not known. Daily activities such as mealtimes and physical exercise may influence timing of seizures. Also, the effects of AEDs on the distribution of seizures remain to be elucidated, as reports disagree (Griffiths and Fox, 1938; Helmchen et al., 1964). Further, anaesthetics may affect seizure frequency, having pro- or anticonvulsant effects (Voss et al., 2008). In our study only 4 of 450 seizures occurred on the same day as the operation. Therefore, anaesthesia probably has no proconvulsive effect relevant to the results of our study.

To sort out the precise effects of exogenous and endogenous factors on epilepsy and seizure distribution, subjects would have to be studied under constant conditions (Duffy and Dijk, 2002). Also, animal studies could provide answers to these questions. This will be the subject of further study.

In the current study, we have taken wakefulness and sleep into account. However, during IEM the normal sleep-wake cycles are difficult to maintain, as the process of implantation and monitoring is fatiguing for patients. Furthermore, as patients remain in bed during IEM, sleep is easily initiated at abnormal times for particular patients. The precise influence of the natural sleep-wake cycle is therefore difficult to interpret in this study.

The exact basis of diurnal patterns in seizure occurrence remains to be elucidated. The circadian rhythm, with the master circadian pacemaker located in the suprachiasmatic nuclei, may play an important role in these seizure patterns. This pacemaker generates and maintains circadian rhythms in many physiological and psychological processes, including

sleep-wake cycle, core body temperature, blood pressure and secretion of several hormones (Hofstra and de Weerd, 2008). It is conceivable that some of these body functions are important to the rhythm in seizure occurrence or even that this pacemaker itself generates a rhythm in seizures. These ideas will be subject of a large prospective study in our epilepsy and sleep centre.

As a whole, our study clearly suggests that seizures do not occur randomly, but seem to take place in daily patterns. Different temporal patterns can be recognized in patterns of seizures from different parts of the brain. Recognition of patterns in seizure occurrence is an important first step in taking the rhythmicity of seizures into account in the diagnosis and treatment of epilepsy. However, more research is needed to further explore these daily patterns and to identify underlying mechanisms that lead to these diurnal rhythms of seizures.

Chapter Six

Chronotypes and subjective sleep parameters in epilepsy patients: a large questionnaire study

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Chronobiology International (2010) 1271-86

Abstract

There is accumulating evidence that epilepsy and seizures may influence circadian rhythms and that circadian rhythms may influence epilepsy. It is also conceivable that seizure timing influences the timing of daily activities, sleeping and wakefulness (i.e. chronotype). Previously only one group has studied the distribution of chronotypes in people with epilepsy, showing significant differences between diurnal activity patterns in two groups of patients with different epilepsy syndromes. To further investigate chronotypes in epilepsy, we performed a questionnaire based study to compare the distribution of chronotypes and subjective sleep parameters (sleep duration and time of mid sleep on free days) in 200 epilepsy patients to the distributions in the general population (n=4042). Within this large group of epilepsy patients, chronotypes of subsamples with well-defined epilepsy syndromes (temporal lobe epilepsy (TLE, n=46), frontal lobe epilepsy (FLE, n=30) and juvenile myoclonic epilepsy (JME, n=38)) could be compared. In addition, twenty-seven patients who had had surgery for TLE were compared with those with TLE who had not had surgery. To determine chronotypes and subjective sleep parameters both the Morningness Eveningness Questionnaire and the Munich Chronotype Questionnaire were used. Significant differences in morningness/eveningness distribution, timing of mid sleep (corrected for sleep duration) and total sleep time on free days were found between people with epilepsy and healthy controls. People with epilepsy were more morning oriented, had earlier mid sleep on free days and sleep duration on free days was longer ($p<0.001$). However, distributions of chronotypes and sleep parameters between the groups of people with TLE, FLE and JME were not found to be different. People who had had surgery for TLE had similar morningness/eveningness parameters and similar sleep durations when compared to those without surgery, but mid sleep on free days was earlier in operated patients ($p=0.039$). In conclusion, this is the first large study focusing on chronotypes in people with epilepsy. We show that the distribution of chronotypes and subjective sleep parameters in people with epilepsy in general is different from that of healthy controls. Nevertheless, no differences are observed between patients with specified epilepsy syndromes, although they exhibit seizures with different diurnal patterns. Our results suggest that epilepsy in general, but not seizure timing, has a significant influence on chronotype behaviour and subjective sleep parameters.

Introduction

Circadian rhythmicity has been demonstrated in many physiological and psychological processes, such as the sleep-wake cycle and hormone production. An important role for well-organized circadian rhythmicity in health and well-being has been shown, including in people with diseases such as cancer (Lamont et al., 2007). Many studies have focused on diurnal rhythmicity in epilepsy. For example, it appears that human seizure occurrence may have 24-h rhythmicity, depending on the origin of seizures (Durazzo et al., 2008; Hofstra et al., 2009a; Hofstra et al., 2009b; Pavlova et al., 2004; Quigg et al., 1998b). However, there is also accumulating evidence that circadian rhythms may also play a role in epilepsy (Hofstra and de Weerd, 2009; Pavlova et al., 2009; Quigg et al., 2000). It is conceivable that the epilepsy syndrome itself and also seizure timing, may strongly influence timing of activity and sleep-wake patterns in people with epilepsy.

The term “chronotype” refers to the preferred phase of an individual for the timing of daily activities, sleep and wakefulness. The distribution of chronotypes is based on the interindividual variation in the endogenous phase of the circadian clock under entrained conditions. Morning types (“larks”) tend to adopt an earlier sleep schedule than evening types (“owls”) and peaks in performance and alertness are seen earlier in the day in morning than in evening types (Horne and Ostberg, 1976; Kerkhof and Van Dongen, 1996; Roenneberg et al., 2004; Roenneberg et al., 2007). These individual preferences for sleep and wake have been studied in relation to epilepsy. In several studies of rodent epilepsy models, daily activity patterns were monitored. One study showed marked changes in these patterns and a delayed acrophase (rhythm peak) for up to 12 weeks after pilocarpine-induced status epilepticus (Stewart and Leung, 2003). In the second study, 24-h behavioural rhythms of rodents with chronic atypical absence seizures were recorded and hyperactivity, but no phase shifts, was observed compared with control rodents (Stewart et al., 2006). A recent study showed that electroconvulsive shocks to rats led to a decreased circadian amplitude of activity, but no phase shifts in locomotor or body temperature rhythms were found (Angles-Pujolras et al., 2009). Another group showed similar results after a single generalized seizure; this resulted in a lower amplitude, but no phase shifts of circadian rest-activity rhythms in Syrian hamsters (Smith et al., 2009). More than half a century ago Janz and Christian described a characteristic sleep-wake cycle in patients with juvenile myoclonic epilepsy (JME, a syndrome of generalized epilepsy) (Janz and Christian, 1957). They observed that these patients have the tendency to get up late in the morning, are most active in the afternoon and evening and fall asleep late at night. One other group has focused on the distribution of chronotypes in people with epilepsy. Pung and Schmitz (2006) compared two groups of patients with different epilepsy syndromes; 20 patients with JME and 20 patients with temporal lobe epilepsy (TLE, a syndrome with focal onset of the seizures in the temporal lobe). They found significant differences between the diurnal activity patterns of the two groups; patients with JME tended to go to bed later at night, to get up later in the morning and to feel fit at a later time during the day than patients with TLE.

An interaction between circadian rhythms and epilepsy may have important clinical consequences for diagnostic and therapeutic procedures. With the existence of such an interaction, it might be beneficial to time clinical observations and administration of drugs appropriately on an individual basis in which circadian rhythmicity is taken into account, so-called chronotherapy. Therefore, we aimed to compare chronotypes of people with epilepsy

to those of the general population. Furthermore, chronotypes of patients with specific epilepsy syndromes (TLE, JME and frontal lobe epilepsy (FLE, a syndrome with focal onset of seizures in the frontal lobe)) were measured and compared. Because of the suspected interaction between circadian rhythms and epilepsy and also because of the differences in seizure timing between the epilepsy syndromes, we expect to find differences in chronotypes between people with and people without epilepsy and also differences in the chronotypes of patients with different epilepsy syndromes.

Material and Methods

Subjects

In this study, self-reported data on morningness/eveningness and sleep-timing of people with epilepsy were analysed. From autumn 2007 until autumn 2009 people with epilepsy from our tertiary epilepsy centre (SEIN) were asked to complete the questionnaires.

Inclusion criteria were: definite epilepsy diagnosis (confirmed in our centre by detailed history and EEG and video registration) and age between 18 and 65 years. Exclusion criteria were: shift work in the last three months and problems that are known to affect circadian rhythms or the sleep wake cycle, such as major depression, bipolar disease or major visual handicaps.

From the group of included patients, data from patients with temporal lobe epilepsy (TLE), frontal lobe epilepsy (FLE) and juvenile myoclonic epilepsy (JME) could be extracted for comparison. An additional group, patients who had had surgery for temporal lobe epilepsy (in which part of the temporal lobe was resected) were also asked to participate. Diagnosis of the epilepsy syndrome, history, co morbidity and current medication could be retrieved from the patient's files. For patient characteristics, see table 1.

Healthy controls were retrieved from a large database from the Chronobiology department of the University of Groningen, the Netherlands (Zavada et al., 2005). Subjects received no financial compensation for participation. The protocol was approved by the institutional Medical Ethics Committee and the study meets the ethical standards of this journal (Portaluppi et al., 2008).

Assessment of chronotypes

Two validated questionnaires were used to determine chronotypes. First, the Dutch version of the Morningness/Eveningness Questionnaire (MEQ) was used. This questionnaire was originally developed by Horne and Ostberg and differentiates morning and evening types (Horne and Ostberg, 1976). It was validated for the Dutch language by Kerkhof (1985). The MEQ contains 19 items that determine when the respondent is most active during the day. Fourteen questions are Likert scale based and five have a time scale divided into hours or 15 minutes. Questions are for instance: "At what time in the evening do you feel tired and as a result in need of sleep?" (time scale) or "If you went to bed at 11 pm at what level of tiredness would you be?" (four item answer). Most questions are preferential, therefore determining the subject's preferences, not the actual timing. Therefore, we added a second questionnaire, the Munich Chronotype Questionnaire (MCTQ). This questionnaire is developed by Roenneberg and colleagues and translated into Dutch by Gordijn (Roenneberg et al., 2003; Roenneberg et al., 2004; Roenneberg et al., 2007; Zavada et al., 2005). Besides questions on employment and shift work, it focuses on actual timing of for instance, going to

bed, falling asleep and waking up. Furthermore, it has the advantage that it explicitly assesses sleep-wake patterns on working days and free days separately.

Data analysis

The MEQs were processed according to the manual, with possible scores ranging from 16 to 86 (16 indicating a definite evening type and 86 a definite morning type). In the MCTQ, total sleep time (or sleep duration) on free days (TSTF, wakeup time – sleep onset time) and mid sleep on free days (MSF) were assessed per patient. To get a better indication of the chronotype the MSF was adjusted by subtracting the difference between the sleep duration on free days and its weekly average if sleep on free days was longer than on working days (MSFsc= uncorrected MSF-(TSTF-average TST)/2). This was done under the assumption that sleep recovery on free days confounds chronotype in a linear way (see (Roenneberg et al., 2007) for an explanation).

MEQ-scores, MSFsc and TSTF were compared between people with epilepsy and healthy controls. These values were also compared in patients with TLE, FLE and JME. Furthermore, a comparison was made between patients who had had TLE surgery (seizure free patients as well as those who continued to have seizures after operation) and the additional TLE group without surgery.

Chronotypes change with age. Most children are early chronotypes, but this pattern changes (delays) as they grow up and in teenagers delayed sleep times are very common. In later adulthood, people tend to become earlier chronotypes again (Roenneberg et al., 2004; Roenneberg et al., 2007). As well as age, gender has also been observed to affect chronotype. In several studies girls and women were found to be significantly more morning oriented than boys and men (Randler, 2007). Because of the possible effects of age and gender on chronotype, we have corrected for both if necessary to rule out as many confounding factors as possible. Differences between categorical variables (e.g. people with epilepsy and healthy controls, men versus women) on MEQ, MSFsc and TSTF data were analysed using unpaired t-tests, ANOVA and multiple linear regression to correct for differences in age between the epilepsy and healthy groups. The regression coefficients (ß's) and proportion explained variance (R^2) are presented with healthy controls as reference group. For comparison of the patient's own opinion on morningness/ eveningness, the χ^2 test was used. Significance was set at *p* level 0.05. For statistical analysis SPSS v12.0.1 (SPSS Inc., Chicago, Illinois, USA) was used.

Results

People with epilepsy versus healthy controls

From 2007 until 2009 two hundred adults with epilepsy (90M/110F; mean age 38.5 yr, median 37 yr, range 15-63 yr) were included in the study; 142 had focal epilepsy; 46 generalized epilepsy and 12 were not (yet) classified. Scores of these patients were compared to those of healthy controls (n=4042, mean age 33.0 yr, median 28 yr, range 15-63 yr). Furthermore, 14 patients who had had surgery for TLE and were seizure free since operation were included for comparison.

Of the 200 patients, 144 took one or two types of anti-epileptic drugs (AEDs), while 51 took three or more types of AEDs. Seventeen different types of AEDs were taken, mostly valproic acid, carbamazepine and lamotrigine (see table 1).

People with epilepsy scored on average 54.3 points on the morningness/eveningness questionnaire compared with an average of 48.2 points for the controls (p<0.001, R^2 = 0.05, age-adjusted difference=5.05, see table 2 and figure 1). This shows that, after correcting for age, the preference of people with epilepsy was significantly more in the direction of morningness compared to the preference of controls. Using the morning/ intermediate/ evening types categories of Horne and Ostberg, the distribution of circadian typology is also different between patients and controls with a clear emphasis on more morningness people in the patient group (p<0.001, see table 2). A significantly earlier mean mid sleep on free days (MSFsc) at 0403h (range 0138-0959h) was observed in the epilepsy group versus 0436h (0028-1130h) in the control group (p<0.001, R^2 = 0.024, age-adjusted difference =-29 minutes). People with epilepsy slept significantly longer on free days than the controls did (8h35m hours versus 8h15m, p<0.001, R^2 = 0.14, age-adjusted difference =33 minutes).

Gender

When comparing 90 male (mean age 39.1 yr, median 37 yr, range 15-63 yr) with 110 female (mean age 39.0 yr, median 37.5 yr, range 15-63 yr) people with epilepsy, no difference in MSFsc was found. Females, however, tended to score higher on the MEQ (p=0.07) and to have a longer sleep duration on free days (8h45m versus 8h23m, p=0.055) than males.

Seizure free patients versus patients with seizures

The comparison between seizure free patients (n=48, 19M/29F; mean age 38.4 yr, median 37 yr) and patients with seizures (n=152, 72M/80F; mean age 38.4 yr, median 37.5 yr) showed no differences in MEQ scores, mid sleep on free days or sleep duration on free days.

Forty six patients were diagnosed with TLE, 38 patients with JME and 30 patients with FLE. Scores of these patients were analysed separately. In addition, the remaining 73 patients (35M/38F; mean age 35.9 yr, median 32 yr, range 21-61 yr; not having TLE, FLE or JME, nor having had surgery) have not been analysed further, as this group was very heterogeneous.

Patients with TLE versus JME versus FLE

Scores of 46 patients with TLE (20M/26F; mean age 39.3 yr, median 38.5 yr, range 15-61 yr) were compared to 38 with JME (13M/25F; mean age 40.8 yr, median 38.5 yr, range 15-63 yr) and 30 with FLE (16M/14F; mean age 39.2 yr, median 39 yr, range 17-63 yr).

No significant differences were found in MEQ, distribution of MEQ-categories, MSFsc or TSTF scores between the groups of patients with TLE, JME and FLE (see table 2 and figure 1). When focusing on the subjective feelings of morningness/eveningness, 52% of people with TLE regarded themselves as morning types versus 55% of those with JME and 50% of those with FLE (p= 0.91).

People with TLE with and without surgery

In addition, patients who had had TLE surgery (n=27, seizure free n=14, not seizure free n=13, mean age 39.7 yr, range 21-61 yr) were compared with the before mentioned group of

46 people with TLE who had not had surgery. MEQ scores were not significantly different between the two groups (see table 2), neither was sleep duration. Time of mid sleep on free days, however, was found to be 33 minutes earlier in those who had had surgery compared with those who had not (p=0.039, unpaired t-test). No significant differences in MEQ, MSFsc or TSTF were found within the surgical group in relation to the presence or absence of seizures.

Concerning subjective assessments on morningness/eveningness, those who had had surgery characterized themselves more as morning types than as evening types (p= 0.028).

Table 1. Subject characteristics.

	Epilepsy patients	Controls	TLE	JME	FLE	Surgery	Other patients[§]
Epilepsy history in yrs (n=patients known)[†]	21.5 (172)	n.a.	19.0 (44)	24.1 (34)	21.9 (27)	28.3[¥] (26)	19.9 (54)
Anti-epileptic drugs		n.a.					
No	5		0	1	1	2	1
1-2	144		39	32	16	15	52
3-5	51		7	5	13	10	20
Number of patients	200	4042	46	38	30	27[‡]	73

[†] The epilepsy history is not clear in all patients

[¥] In patients that are seizure free since operation, epilepsy history is the time from year of first seizure until year of operation

[§] The patients not belonging to the TLE, JME or TLE group, nor to the surgery group

[‡] 14 of the 27 patients were seizure free

Table 2. Distribution of chronotypes and sleep parameters. Data is expressed as mean ± standard deviation.

	Epilepsy patients	Controls	*p*	TLE	JME	FLE	*p*	TLE	Surgery	*p*
MEQ	54.3 ± 8.2 [34-80]	48.2 ± 12.0 [17-81]	***	55.0 ± 8.5	52.9 ± 6.7	52.4 ± 9.0	*ns*	55.0 ± 8.5	57.3 ± 9.4	*ns*
Circadian type [¥]			***				*ns*			*ns*
n= M	63	876		15	10	10		15	13	
n=I	125	1927		30	26	16		30	11	
n=E	12	1239		1	2	4		1	3	
MSF	0403 ± 1:07	0436 ± 1:15	***	0410 ± 1:07	0403 ± 0:50	0410 ± 1:38	*ns*	0410 ± 1:07	0337 ± 0:59	*0.039*
TSTF	8:35 ± 1:19	8:15 ± 1:25	***	8:32 ± 1:18	8:40 ± 1:02	8:13 ± 1:20	*ns*	8:32 ± 1:18	8:28 ± 1:01	*ns*
Subjective M/ E type ‡				24/ 22	21/ 17	15/ 15	*ns*	24/ 22	20/ 7	*0.028*
Number of patients	200	4042		46	38	30		46	27	

[¥] M Morning; I Intermediate; E Evening, as determined by the MEQ; ns not significant; *** $p<0.001$.

‡ Question 19 of the MEQ: “One hears about “morning (M) types” and “evening (E) types”. Which one of these types do you consider yourself to be?”

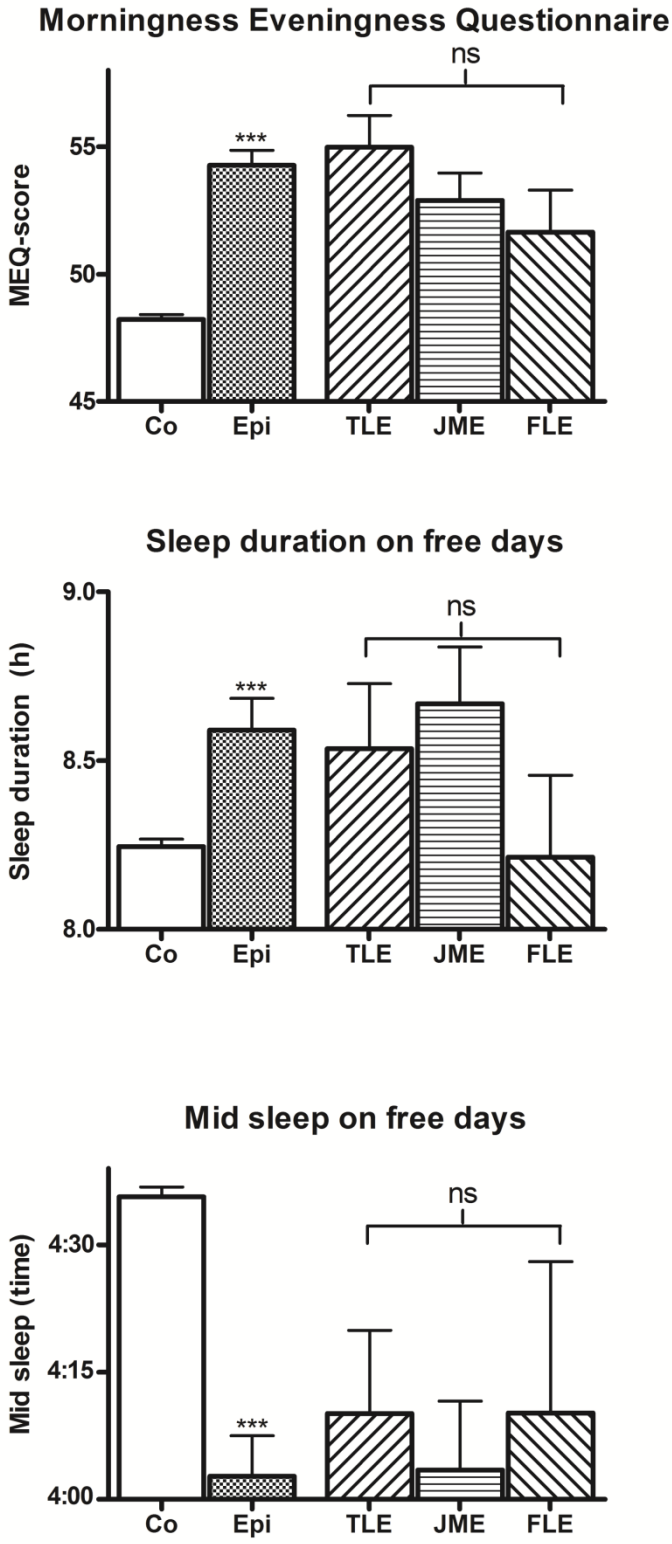

Figure 1. The distribution of MEQ-scores (top), total sleep time on free days (mid) and mid sleep on free days (bottom) in epilepsy patients versus healthy controls and in patients with temporal lobe epilepsy, juvenile myoclonic epilepsy and frontal lobe epilepsy. Co controls; Epi epilepsy; TLE temporal lobe epilepsy; JME juvenile myoclonic epilepsy; FLE frontal lobe epilepsy; h hour, ns not significant; *** p<0.001.

Discussion

The main findings of this study were that chronotypes and subjective sleep parameters of patients with epilepsy significantly differ from healthy individuals, with people with epilepsy being more morning oriented, which is also reflected in having an earlier mid sleep on free days. They also sleep longer on free days than people without epilepsy. In contrast, when focusing on three different patient groups, it was observed that chronotypes and subjective sleep parameters between patients with TLE, FLE or JME are similarly distributed.

From literature, there is evidence that there might be a relationship between circadian rhythmicity and epilepsy. Circadian rhythms in several physiological functions, such as heart rate variability, have been shown to be changed in people with epilepsy (for further review, see (Hofstra and de Weerd, 2009)). Also, several studies in humans have shown diurnal rhythmicity in seizure occurrence (Durazzo et al., 2008; Hofstra et al., 2009a; Hofstra et al., 2009b; Pavlova et al., 2004; Quigg et al., 1998b). Unfortunately, none of these studies have taken circadian rhythmicity into account. Others have focused on circadian influence, for instance Quigg et al have shown circadian rhythmicity in spontaneous seizures in a rat model of mesial temporal epilepsy and recently, evidence for circadian rhythmicity in interictal discharges was provided using a forced desynchrony protocol in a small number of patients with generalized epilepsy (Pavlova et al., 2009; Quigg et al., 2000).

As mentioned, there are some animal studies in which daily activity patterns were studied. Nevertheless, these studies do not agree on whether seizures or electroshocks result in a phase shift in behavioural rhythms and in what direction the amplitude is altered (Angles-Pujolras et al., 2009; Smith et al., 2009; Stewart et al., 2006; Stewart and Leung, 2003). The influence of seizures on other circadian rhythms has also been studied. For example, postictal phase shifts in core body temperature in a rat model of temporal epilepsy have been observed, although studies are not consistent (Quigg et al., 2001). In humans, a postictal phase shift was observed in the circadian rhythm of melatonin release, although, again, the results from different studies are not conclusive. Some authors describe low baseline levels of melatonin in people with epilepsy, whilst others found elevated levels (Bazil et al., 2000; Fauteck et al., 1999; Laakso et al., 1993; Molina-Carballo et al., 1994; Schapel et al., 1995). A recent study found a normal plasma melatonin curve in epilepsy patients under dim lit conditions (Pavlova et al., 2009). Likewise, elevated levels during or directly after complex partial seizures and generalized tonic clonic seizures have been described, whilst other authors observed no changes after complex partial seizures and generalized tonic clonic seizures (Bazil et al., 2000; Molina-Carballo et al., 2007; Rao et al., 1989). A phase shift caused by seizures might be part of the explanation why chronotypes in epilepsy patients differ from healthy subjects. In this study, we have compared results of 48 patients that were seizure free and 152 that remained to have seizures and found no differences in chronotypes or subjective sleep parameters. Seizure frequency in the patients varied from once a year to several seizures a day. Also, seizure severity varies between patients, from having simple partial or absence seizures to having generalized tonic clonic seizures. For this reason, it is difficult to perform correct correlation of chronotype and sleep parameters with the presence and severity of seizures.

Another finding of the present study that there is no significant difference in chronotype distribution between the three different patient groups is in contrast to one previously published study. In this study, it was observed that patients with JME tended to go to bed

later at night, to get up later in the morning and to feel fit at a later time during the day compared to age matched patients with TLE (Pung and Schmitz, 2006). JME patients also characterized themselves as evening types more than did those with TLE. The earlier observations of Janz and Christian in the 1950s also showed this characteristic eveningness pattern in JME patients, but these findings were not endorsed by objective measurements (Janz and Christian, 1957). In contrast to findings of these two studies, we have shown that chronotypes of patients with TLE and JME do not differ and that JME patients cannot be characterized as evening types more than the other groups, nor do they classify themselves more as evening than morning types. The discrepancies between studies are not easily explained. The subjects in the present study were somewhat older (~40 yr on average compared to ~34 yr in the study of Pung & Schmitz, 2006) and as mentioned, age has large influences on circadian rhythmicity and sleep parameters (Randler, 2008; Roenneberg et al., 2004; Roenneberg et al., 2007; Tonetti et al., 2008). However, it seems unlikely that this is the only explanation for the large difference in findings. As raised before, in healthy people the largest shift in chronotype occurs around the age of 20 yr (Roenneberg et al., 2004). Furthermore, it is not very likely that criteria to diagnose TLE or JME were different. Also, exclusion criteria in the two studies were alike. In both studies, sample sizes were relatively small, this could be part of the explanation. Unfortunately, more literature on this matter in humans is not known.

Seizure occurrence is also an important factor in sleep. There are numerous studies that have shown that sleep can be severely disrupted in people with epilepsy. For example, patients with partial epilepsy report a twofold higher prevalence of sleep disturbance than controls (de Weerd et al., 2004). The severity of sleep disturbance depends on type of epilepsy syndrome, frequency of seizures, type of seizures and other underlying (neurological) deficits (Sammaritano and Therrien, 2002). For example, sleep abnormalities are more common in patients with generalized epilepsy than in patients with focal epilepsies. Most studies on the effects of epilepsy, seizures or AEDs on sleep were performed with polysomnography to determine objective sleep parameters. These objective measurements of sleep characteristics cannot be directly compared to the results of our questionnaire study. However, we were able to compare total sleep time of patients with generalized epilepsy (JME) to the TSTFs of patients with focal epilepsy (TLE and FLE) and also of patients with and without seizures and no differences between these parameters were observed.

It is conceivable that seizure timing has a strong influence on the activity/ sleep/ wake pattern of patients. In a previous study, we recorded 450 seizures detected by intracranial EEG. These seizures showed an uneven distribution over the day, depending on the lobe of origin: temporal lobe seizures occurred preferentially between 1100 and 1700h, frontal seizures between 2300 and 0500h and parietal seizures between 1700 and 2300h (Hofstra et al., 2009b). Other studies showed roughly similar time peaks in temporal and frontal seizures (Durazzo et al., 2008; Hofstra et al., 2009a; Hofstra et al., 2009b; Pavlova et al., 2004; Quigg et al., 1998b). In contrast, many studies have shown that in patients with JME most seizures occur on awaking (Janz and Christian, 1957). As only part of the patient population of this study overlaps with the patient population included in our previous study (Hofstra et al., 2009b) we cannot state that seizure occurrence in the TLE and FLE groups is exactly similar as we have found in that previous study. However, assuming that in our subjects seizures occur in similar diurnal rhythms as shown in these previous studies, the hypothesis of an interaction between seizure timing and chronotype could thus be tested. We

compared the three groups of people with TLE, FLE and JME with presumed seizure peaks at different times of the 24-h day (the afternoon, during the night and after awaking). The conclusion from thorough comparison nevertheless must be that having seizures at different times of day in the different patient groups does not appear to be related to chronotype, as reflected neither by preference for morning/evening type behaviour nor by mid sleep on free days, nor to sleep duration on free days.

In our study, AEDs are taken by all but five patients. Sleep duration can also be influenced by AEDs, but the effects of the different drugs vary substantially (Foldvary-Schaefer and Grigg-Damberger, 2006). As mentioned, most patients use valproic acid, carbamazepine and lamotrigine. According to literature long-term usage of valproic acid has no reported effects on sleep duration (Foldvary-Schaefer and Grigg-Damberger, 2006). Also, no influence on sleep duration was observed with carbamazepine or lamotrigine (Foldvary et al., 2001; Gigli et al., 1997). In our study, we observed that the mean sleep duration on free days in patients with epilepsy was longer than in healthy controls, mainly due to an earlier sleep onset time. As the three most frequently used AEDs do not have effects on sleep duration, it is not likely that the use of these drugs is the sole factor for this difference. A reason for a longer TSTF could be that people with epilepsy try to compensate for sleep of lower quality by sleeping longer. Timing of taking the AEDs is another important factor, as many of these drugs have sedative effects. We could not include an analysis on the times that patients take their medication in the current study, but this study is in progress in our centre.

Apart from possible effects on sleep, these drugs may very well have effects that obscure the endogenous component of circadian rhythms, so-called masking effects, like is known from NSAIDs and beta-blockers (Murphy et al., 1996; Stoschitzky et al., 1999).

A factor that is known to have large influence on circadian rhythmicity is age (Roenneberg et al., 2004; Roenneberg et al., 2007; Tonetti et al., 2008). The mean age of the patient and control group was respectively 38,5 and 33 years and median age was 37 and 28 years. These differences in age could be contributing to the difference in morningness/eveningness. However, as mentioned before, the largest shift in chronotype occurs around the age of 20 yr (Roenneberg et al., 2004).

Also differences in daily habits, such as employment or education and light exposure are important factors. However, this is not part of the MEQ and in the Dutch MCTQ only employment and light exposure are mentioned. As a consequence, how many adults were employed can be determined, but not how many children and young adults went to school. Therefore, a fair correlation cannot be made. Further, light exposure on working days and free days is part of the Dutch shortened MCTQ, but because many of our patients have indicated that they were uncertain about how many hours of light they were exposed to, we have not included this in our analysis.

To study chronotypes we have used two validated questionnaires. The MEQ, which we used, was recently described as a fair predictor of the endogenous circadian phase or period (Sack et al., 2007). In addition to this well-known and widely used MEQ, the MCTQ was used. From the MCTQ mid sleep on free days (sleep without social obligations) can be extracted. From previous studies is known that the MEQ-score and MSF are strongly correlated (Zavada et al., 2005). Furthermore, studies have shown that the circadian melatonin rhythm (specifically the melatonin onset in dim light or DLMO) correlates strongly with sleep midpoint and MSF is therefore also a good predictor of the circadian phase of

normal, healthy young adults (Martin and Eastman, 2002). The MSF reflects a phase marker under entrained conditions. Together, these two questionnaires give a fair representation of the subject's chronotypes (Zavada et al., 2005). Furthermore, explicit inclusion and exclusion criteria were used to ensure that confounding factors were minimised.

Several factors limit strong conclusions in this study. First, questionnaires were used, therefore our data is subjective. To further explore the observed differences in chronotype and sleep parameters, it would be valuable to include sleep records, as this could add objective information. Furthermore, seizure diaries of epilepsy patients or even continuous EEG and video monitoring could provide detailed knowledge on how seizure frequency and severity influences chronotypes and sleep parameters. Also, further studies on the effects of AEDs and timing of taking medication on circadian rhythmicity and sleep would be worth studying. Furthermore, we have not assessed physiological rhythms of our subjects under study. For further research on the differences in chronotype and sleep parameters between people with and without epilepsy, this might be an important focus.

This study was designed to elucidate the relationship between chronotype and different forms of epilepsy. Also, we aimed to shed light on the influence of seizure timing on chronotype. Our hypothesis was that the distribution of chronotypes would be different in people with epilepsy, as many circadian rhythms have found to be changed in epilepsy patients and because of the expected influence of different temporal seizure patterns on the diurnal activity pattern and sleep-wake cycle. The current study has provided the novel finding that the distribution of chronotypes is different in people with epilepsy compared to those without epilepsy. However, no differences between patients with different epilepsy syndromes have been observed. This suggests that epilepsy in general, but not specific epilepsy syndromes with different seizure patterns over the day have great influence on the chronotype. Subsequent studies have to elucidate further what causes the difference in chronotypes in epilepsy patients. Clear individual differences in chronotype behaviour are found in people with epilepsy as were previously found in healthy controls. This means that it could be beneficial to time clinical observations and administration of drugs appropriately on an individual basis. This so-called chronotherapy could be a future improvement of the care for people with epilepsy. This is currently been assessed in our centre.

Chapter Seven

Morningness and eveningness: when do epilepsy patients take their drugs?

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Submitted

Abstract

Almost one third of epilepsy patients remain to have seizures despite adequate drug treatment. Chronotherapy (based on dynamic changes in drug pharmacology and disease-related processes) could be a promising new treatment option. We aimed to explore whether different circadian types (i.e. morning, intermediate and evening types) already adjust administration times of their anti-epileptic drugs (AEDs) as a first step in exploring chronotherapeutic possibilities. Therefore, we performed a questionnaire based study to compare the behaviour of different circadian types in relation to the times of taking drugs. Circadian type was determined by the Morningness/Eveningness Questionnaire. Results clearly show that morning types are taking their AEDs significantly earlier than do evening types on free days, that is 100 minutes earlier for the morning dose (p<0.001) and 55 minutes earlier for the evening dose (p=0.019). Also, times of taking AEDs in the morning on work days differ significantly between morning and evening types (55 minutes, p<0.001). Regardless of circadian type, drugs on free days are taken significantly later than on working days, which is most pronounced in evening types (up to 90 minutes delay in the evening types, p=0.005). Age and gender did not influence times of taking AEDs. In conclusion, this is the first study to show that patients adapt times of taking medication to their circadian type.

Introduction

Almost one third of epilepsy patients continue to have seizures despite adequate drug treatment (anti-epileptic drugs, AEDs) (Kwan and Brodie, 2000; Panayiotopoulos, 2007). Besides the development of new drugs and other treatment options, it is also important to investigate how current AEDs can be used better to improve seizure control. Chronotherapy, which is delivery of therapy based on the dynamic changes in both drug pharmacology and in disease-related processes (Levi and Schibler, 2007), could be a new option.

Chronotherapy is already used in various diseases, such as cancer, pulmonary disease (e.g. allergic rhinitis and bronchial asthma), treatment of pain and cardiovascular diseases (Levi and Schibler, 2007; Prisant, 2004; Smolensky et al., 2007). Several studies have shown that administration of drugs adjusted to the circadian rhythm can be successful, not only in reducing symptoms, but also in reducing adverse drug effects and thereby improving quality of life (Mormont and Levi, 2003). Whether chronotherapy will be successful depends on several factors, i.e. the circadian differences in pharmacodynamics of the specific treatment and the 24-h pattern in manifestation and intensity of symptoms in a disease. In epilepsy, various AEDs are being used, with different pharmacodynamic profiles. Also, it has been shown in recent human studies that seizures may occur in different 24-h patterns (Durazzo et al., 2008; Hofstra et al., 2009a; Hofstra et al., 2009b; Pavlova et al., 2004; Quigg et al., 1998b). Therefore, there is good reason to believe that chronotherapy could improve seizure control. To our knowledge, only one group has investigated this option in epilepsy patients. Yegnanarayan and others changed administration times in patients with diurnal seizures that were not controlled by phenytoin and/or carbamazepine (Yegnanarayan et al., 2006). The authors found that with adjusting administration times from 0800h to 2000h therapeutic drug levels were achieved more easily and toxic manifestations were reduced. Until now, no other epilepsy studies have been published on adjusting therapy to a person's circadian type or chronotype, whilst large differences between individual chronotypes exist.

Before studying whether adjustment of medication administration times leads to improved seizure control, the spontaneous behaviour of patients towards these times has to be studied. For instance, in our tertiary centre patients are described AEDs with the advice to take these drugs at certain times. These times are 0800h, 1200h, 1800h and 2300h, depending on the frequency per day. However, it is conceivable that an extreme morning type who gets up at 0600h in the morning tends to take the medication far before 0800h and someone that is used to getting up late will take medication later than the advised time. Therefore, this study focuses on the moments that patients take medication in practice. We hypothesize that these times vary strongly between morning, intermediate and evening types.

Methods

Subjects

Patients from the nationwide epilepsy centre SEIN were approached to complete a questionnaire to determine their circadian type and to fill in the average times when they take their AEDs on work or school days and free days separately.

The study was approved by the institutional Medical Ethics Committee. Subjects were not paid for participation.

Assessment of circadian type

A validated questionnaire was used to determine the patient's circadian type. This questionnaire was the Morningness/Eveningness Questionnaire (MEQ). This has originally been developed by Horne and Ostberg and differentiates Morning, Intermediate and Evening types (Horne and Ostberg, 1976) and was validated for Dutch language by Kerkhof (Kerkhof, 1985).

Data analysis

The MEQs were processed according to the manual, with possible scores ranging from 16 through 86 (with 16 expressing a definite evening type and 86 a definite morning type). The group was split into three groups according to MEQ scores: evening types (16-41), intermediate types (42-58) and morning types (59-86). Times of taking medication in the morning and evening on work or school days and free days were compared between the three groups. Furthermore, differences between taking AEDs on work days and free days were assessed. Gender and age differences were also determined.

To compare times of taking medication on work versus free days within the groups paired t-tests were used and to compare gender differences the unpaired t-test was applied. Differences in timing between the morning, intermediate and evening types were analysed using the ANOVA test with Tukey's Honestly Significant Difference post hoc test. Because of the age differences between the groups linear regression was also applied to correct for these age differences. Furthermore, effect modification by age (younger half of the group versus older half of the group) was assessed. Significance was set at p level 0.05. For statistical analysis SPSS v17 (SPSS Inc., Chicago, Illinois, USA) was used.

Results

Two hundred and eight adult patients (97M/111F; mean and median age 37, range 15-64) were included in the study. All patients were using AEDs.

According to the MEQ-scores the group was divided into morning types (n=67), intermediate types (n=122) and evening types (n=19). For further characteristics, see table 1.

Anti-epileptic drugs

Eighty three subjects were on monotherapy, 84 used two different AEDs and 41 used more than two different AEDs (34 used three, six patients used four and one patient used five different AEDs). Most frequently used AEDs in this population were valproic acid (39%), carbamazepine (36%) and lamotrigine (36%), followed by levetiracetam (22%). There was no difference in the number of AEDs taken by morning, intermediate or evening types. Nineteen patients took AEDs once a day, 122 patients took them two times a day, 46 were on a thrice-a-day schedule and 21 patients were on a four-times-a-day schedule. As most patients were taking medication in the morning (prescribed on 0800h, n=188) and early evening (prescribed on 1800h, n=161), this data was analyzed.

Table 1. Characteristics of the patient population

	Morning types	*Intermediate types*	*Evening types*	
Age (median) in yrs	27.8 (27.0)	35.3 (32.0)	43.4 (44.5)	
Gender	27 M; 40 F	61 M; 61 F	9 M; 10 F	
Mean MEQ-scores	64.3 [59-75]	51.4 [42-58]	37.3 [28-41]	
Total	*67*	*122*	*19*	*208*

Table 2. Mean times of taking anti-epileptic drugs (in hours)

	Morning types	*Intermediate types*	*Evening types*
Working days	0729 [0600-0900]	0743 [0545-1030]	0824 [0730-1000]
	1800 [1400-2150]	1814 [1600-2100]	1832 [1700-2000]
Free days	0814 [0700-1100]	0859 [0630-1300]	0954 [0800-1300]
	1807 [1600-2130]	1829 [1615-2300]	1902 [1700-2200]

Differences between morning, intermediate and evening types

In the morning type group, the average times of taking medication in the morning on work or school days was 14 minutes (p=0.006) earlier than in de intermediate group and 55 minutes earlier than in the evening type group (p<0.001). The times of taking evening medication did not differ between morning, intermediate and evening types on work days (see table 2 and figure 1).

Significant differences were seen in times of taking medication in the morning when the individual was free. Morning types took their AEDs 45 minutes earlier than intermediate types (p=0.004) and 100 minutes earlier than evening types (p<0.001). Times for the evening dosage only differed between morning and evening types (55 minutes, p=0.019, see table 2 and figure 1).

Differences between work days and free days

In the morning group patients delayed taking their morning medication on free days by 41 minutes (p<0.001), the intermediate types took their medication 76 minutes later (p<0.001), whilst evening types delayed their morning dose by 90 minutes (p=0.005), in comparison to work days (see figure 2). In the evening taking medication was also later on free days than on working days in the intermediate group (15 minutes, p<0.001), but not in the morning and evening group.

In the 208 subjects, regardless of morningness or eveningness, administration times in the morning were on average 68 minutes later on free days than on work days (0742h vs 0850h, p<0.001). Times for the evening dosages also differed (1811h vs 1826h, p<0.001).

No differences were seen when comparing the administration times of female and male patients. Also, there was no effect modification by age in the relationship between chronotype and the time the AEDs were taken.

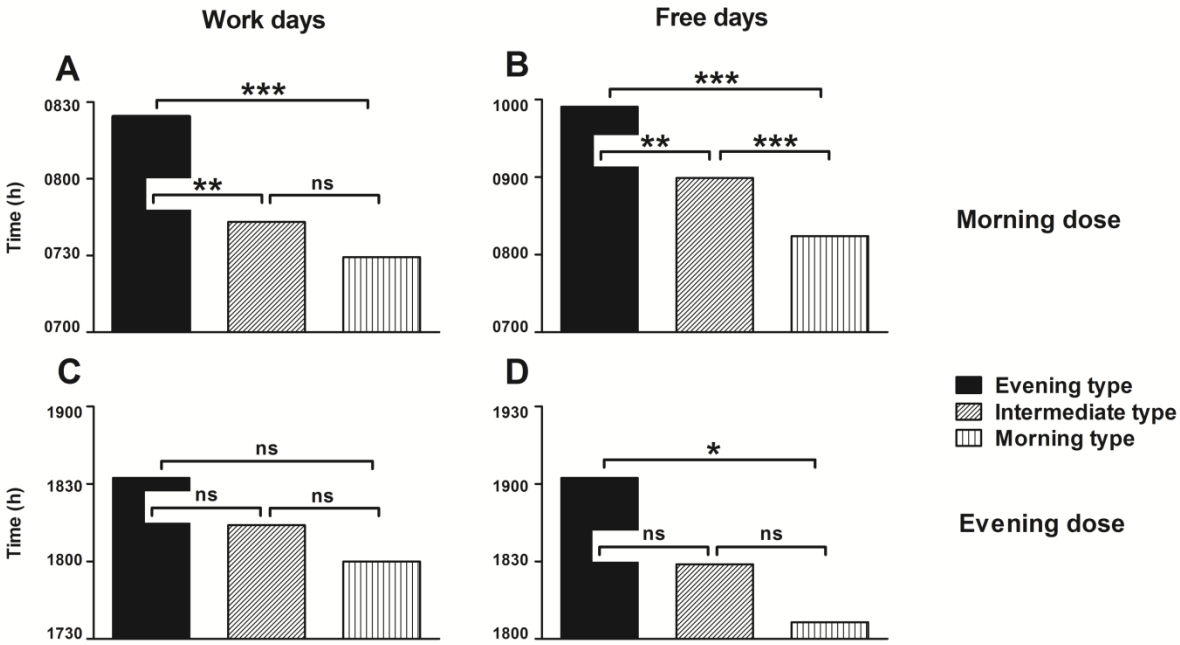

Figure 1. Bar graph showing the mean timing of drug administration in different circadian types in the morning on work days (A) and free days (B). Timing of evening dose is shown in C (work days) en D (free days).

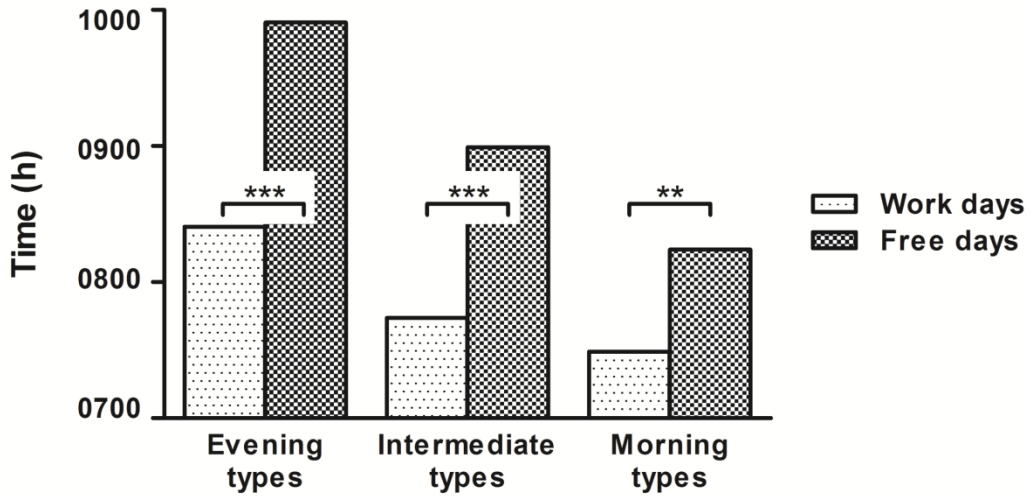

Figure 2. Bar graph showing the difference in mean timing of drug administration in the morning comparing work and free days in evening, intermediate and morning types.

Discussion

The main finding of this study is that epilepsy patients adapt their drug administration times to their circadian type. On free days, morning types take their AEDs significantly earlier than do evening types, with most pronounced differences in the morning doses. Also, times of taking AEDs in the morning on work days differ between morning and evening types. Furthermore, in general, drug administration times are significantly delayed on free days compared to work days.

Many epilepsy patients remain to have seizures with the current drugs. Amongst others, chronotherapy may offer a solution to drug-resistant epilepsy. Although chronotherapy is being used in various other disorders, it is not (yet) being applied in epilepsy. However, before chronotherapeutic options in epilepsy can be explored, one needs to know at what times drugs are used by patients in practice. To our knowledge, this is the first study focusing on the influence of circadian typology in drug administration times. We have included a large group of morning, intermediate and evening types to see whether patients adapt these administration types to their biological preferences in daily activities and sleep-wake cycle. By means of a widely used and validated questionnaire we have assessed the individual's circadian type and correlated this to the individual's drug administration times.

There are some limits to this study. One has to realise that filling in the times of taking medication is subjective. Patients might not be completely honest and fill in what they think is appropriate. This would mean that in reality administration times would vary even more. Furthermore, our evening type group is rather small in comparison to the two other groups.

As results of this study show that patients take their AEDs adapted to their level of morningness/ eveningness, physicians need to realize this when prescribing drugs to their patients. In itself, this adaptation to the circadian type does not pose a risk. However, if drug administration times on free days are significantly delayed compared to work days, like in evening types on average by 90 minutes, this irregularity could contribute to poor seizure control. Therefore, in case of poor seizure control, it may be helpful to ask patients to fill in their drug administration times on work days and free days, as we have done in this study, to discover such irregularities. Furthermore, it would be valuable to emphasize the importance of taking drugs at the same time every day, regardless of whether it is a free day or work day.

Modern day society is built around morning and intermediate types. Schools start in the early morning and the same goes for most jobs. Therefore, many people have to adapt their sleep-wake cycle to society. As intermediate and evening types go to bed later than do morning types, but still have to rise early, they often sleep too little during the week. This can lead to a sleep debt, the cumulative effect of not getting enough sleep, built up during the week. Therefore, it makes sense that intermediate and evening types get up later on free days than on work days for two reasons. First, because of their biological clock and second, because of catching up with lost sleep. This, in turn, causes the larger differences between taking morning medication on work days and free days in comparison to morning types.

In conclusion, we have shown that there is morningness/eveningness in the times at which patients take their medication, which means that patients adapt these times to their circadian type. Also, results show that patients delay times of taking medication on free days significantly compared to work days. Further research is needed to see whether these findings are confirmed when circadian rhythmicity is measured in epilepsy patients by for

example the melatonin curve or core body temperature (Hofstra and de Weerd, 2008). Furthermore, correlation of seizure occurrence and seizure severity to timing of AEDs in different circadian types can show whether adaptation of drugs to the individual circadian rhythm might improve seizure control. A step further would be to consistently adjust the drug administration times to the patient's circadian or chronotype to see whether this decreases seizure frequency, severity and adverse effects.

Chapter Eight

Timing of temporal and frontal seizures in relation to the circadian phase: a prospective pilot study

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In revision

Abstract

There is strong evidence that epileptic seizures occur in diurnal patterns. A study in rat models of partial epilepsy showed circadian seizure patterns and in humans circadian rhythmicity in interictal discharges has been found, suggesting that circadian rhythm may play a role in epilepsy. Circadian influences on human seizure patterns have not been investigated. We performed a pilot study to ascertain influences of the circadian rhythm on seizure occurrence. We prospectively outlined circadian rhythms of patients admitted for long-term EEG and video monitoring, using measurement of the dim light melatonin onset (DLMO). Seizures during admission were recorded with continuous EEG and video monitoring. The DLMO ranged from 1846h to 2313h (mean 2122h). One hundred and twenty-four seizures of 21 patients were analysed. Seizures of temporal lobe origin occurred mainly between 1100 and 1700h and frontal seizures were seen mostly between 2300 and 0500h. When correlating seizure timing to the individual circadian phase as measured by the DLMO, the following was seen: temporal seizures occurred most frequently in the six hours before DLMO and frontal seizures mainly in six to twelve hours after the DLMO. The results of this pilot study suggest that temporal and frontal seizures not only occur in diurnal patterns, but are time locked to the circadian phase.

Introduction

Circadian rhythms are endogenously mediated 24-h cycles of physiological and psychological processes, including the sleep-wake cycle, core body temperature, blood pressure, task performance and hormone production (Hastings et al., 2007). The master circadian pacemaker can be found in the suprachiasmatic nuclei (SCN) in the anterior hypothalamus. An important role for well-organized circadian rhythmicity in health and well-being has been shown, including in people with diseases such as cancer (Lamont et al., 2007). There is accumulating evidence that circadian rhythms may also play a role in epilepsy (Hofstra et al., 2009a; Hofstra and de Weerd, 2009; Pavlova et al., 2009; Quigg et al., 2000).

Several studies have shown diurnal rhythmicity in seizure occurrence, that seems to depend on origin and type of seizures (Durazzo et al., 2008; Hofstra et al., 2009a; Hofstra et al., 2009b; Pavlova et al., 2004; Quigg et al., 1998b). None of these studies has taken circadian rhythmicity into account. To date, only one study has focused on circadian rhythmicity and provided evidence for circadian rhythmicity in interictal discharges using a forced desynchrony protocol, in which internal circadian time can be desynchronized from external time, in a small number of patients with generalized epilepsy (Pavlova et al., 2009).

In animal studies, strict protocols can be used to uncover the circadian rhythm and investigate a possible interaction in more detail. One study showed that during the light-dark exposure, spontaneous limbic seizures in an epileptic rat model occurred in statistically non-uniform patterns with nearly twice as many seizures present during the light portion (Quigg et al., 2000). During the period of constant darkness, allowing free running circadian rhythmicity, seizures continued to occur in the same pattern observed during the light-dark episode when referenced to the circadian rhythm of these rats, suggesting that spontaneous limbic seizures recur in a true endogenously mediated circadian pattern.

An interaction between circadian rhythms and epilepsy may have important clinical consequences, as it might be beneficial to time diagnostic procedures and administration of drugs appropriately on an individual basis in which circadian rhythmicity is taken into account, so-called chronotherapy. Underlying mechanisms of seizure occurrence can also be further studied. Altogether, there is evidence for circadian rhythmicity in seizures, but this has not yet been confirmed in humans. We performed a pilot study in which circadian phase of inpatients was determined by measurement of the dim light melatonin onset (DLMO) and circadian phase was correlated to seizure occurrence.

Methods

Subjects

In our tertiary epilepsy and sleep centre, patients are admitted for continuous EEG and video monitoring, performed for diagnosis and classification of epilepsy or for presurgical evaluation. Patients are monitored in a specially designed unit. Monitoring is performed according to established recommendations (Velis et al. 2007). EEG and video monitoring is performed continuously, sometimes over many days. Specially trained nurses are always present in the living room, while patients are constantly monitored by EEG and video with real-time human supervision when in their bedrooms.

The study was approved by the institutional Medical Ethics Committee. Patients admitted for EEG-monitoring of at least 72 hours were asked to participate in the study. All subjects gave written informed consent. Subjects had no serious co-morbidity or visual handicaps and had not performed shift work or travelled more than one time zone in the month prior to the study.

Assessing circadian rhythm

For delineating the individual circadian rhythm the dim light melatonin onset (DLMO) was used. Melatonin concentrations were measured in saliva samples that were collected by chewing on a salivette (Sarstedt, Nümbrecht, Germany) for approximately one minute. Fifteen minutes prior to saliva collection the mouth was rinsed with water and eating or drinking was not allowed until after collection. Saliva collection occurred at 1800h and hourly from 2000-2300h and at 0200h and 0400h on the first and third evenings of admission. Subjects remained seated 15 min prior to collection as posture can influence melatonin concentration (Deacon and Arendt, 1994). Light intensities never exceeded dim light (<50 lx) from 1 hour before until the end of the saliva collection periods (Lewy et al., 1999). Also, during the collection period consumption of caffeine, bananas or drinks with artificial colouring was not allowed as this could contaminate the saliva samples (Gordijn et al., 1991). During the day patients remained in normal indoor lighting conditions (a living room with artificial light and daylight through windows). Subjects abstained from smoking. Saliva samples were kept at 4ºC until the individual experiment ended. Subsequently, the samples were sent to the Gelderse Vallei Hospital in Ede, the Netherlands, where they were stored at -20ºC until assayed. Before assay the samples were centrifuged (1000g, 5 minutes). Melatonin levels in saliva were measured by a commercially available RIA kit (Bühlmann Laboratories AG, Switzerland). Aliquots of 400 µl of the saliva sample were added directly to the assay tubes. The detection limit of the assay was 0.5 pg/ml sample.

The DLMO (the time when melatonin concentration starts rising in the evening) was used as a marker of the circadian phase. The DLMO was calculated per subject, defined as the time at which the melatonin concentration crossed the absolute threshold of 3 pg/ml (Benloucif et al., 2008). DLMO was determined by linear interpolation between the two samples around the value of 3 pg/ml (for an example, see figure 1).

The light-dark cycle has large influence on the circadian rhythm, as it is a major factor in resetting the circadian system (so-called entrainment). It is therefore important to control for this factor. As we wanted to be certain of equal entrainment of the subjects, the DLMO of the third evening in the clinic was used. The DLMO of the first night was used for comparison and back-up.

EEG and video recording systems

EEG monitoring was performed by a 40-channel Lanotta long term monitoring amplifier in combination with a Stellate Harmonie Epilepsy Monitoring System. Scalp recordings were performed using the 10-20 system with additional electrodes F9, F10, P9 and P10 and other positions as needed, all according to the 10-10 system. Electromyography (submental), electro-oculography and electrocardiography electrodes were also used. Respiration was measured by abdominal piezo respiratory effort sensors. Video monitoring was performed simultaneously by closed circuit television and recorded from multiple remotely controlled Axis cameras. All coupled EEG and video data was saved. After interpretation all material containing important clinical information was archived (Hewlett Packard).

Data analysis

All EEGs and videos were evaluated by well-trained technicians from the clinical neurophysiology department and by a board certified clinical neurophysiologist. In case of discrepancy, the interpretation of another clinical neurophysiologist was decisive. A seizure was defined as an electroencephalographic epileptic discharge of at least ten seconds. Nearly all EEG events also had clinical correlates. A seizure cluster was considered one seizure, with the starting point of the first seizure as the start point. Patients with more than five seizures per time bin (see below) on a given day were excluded, as this could lead to erroneous peaks in seizure occurrence.

To correlate seizure with circadian time, seizures times were related to the DLMO. The DLMO was defined as midpoint, with bins of 12 to 6 hours (bin I) and 6 to 0 hours before DLMO (bin II) and 0 to 6 hours (bin III) and 6 to 12 hours after DLMO (bin IV). For example, a seizure at 2000h in a patient with a DLMO at 2030h, was analysed in bin II.

To correlate seizures with clock time, the day was divided into four bins, as was done in previous studies (Hofstra et al., 2009a; Hofstra et al., 2009b). With the general accepted nadir of the core body temperature as a starting point, these time bins were 0500-1100h (I), 1100-1700h (II), 1700-2300h (III) and 2300-0500h (IV), consecutively.

The non-parametric binomial test was used to test whether numbers of seizures in the four time bins were significantly different from a uniform distribution. When assuming whether seizures occur randomly, a percentage of 25% of the total number of seizures in each of the four bins is expected. The binomial test measures differences between the expected percentages and observed percentages. For statistical analysis SPSS v17 (SPSS, Chicago, IL, U.S.A.) was used. Significance was set at *p* level 0.05.

Results

Subjects

Over two years 21 subjects were included (13M/ 8F, mean age 38.6 years, range 18-62, mean duration of registration 113h, range 72-288h, median 96h). Subjects had on average 6 seizures per registration (median 5, range 2-29). In two subjects an additional registration was performed as no seizures were recorded during the first monitoring period. In another two subjects the melatonin curve of the third night could not be analysed due to missing values (i.e. not enough material for analysis) and therefore the DLMO of the first night was used. The mean DLMO in this population was 2122h, ranging from 1846h to 2313h.

Seizures

A total of 124 spontaneous seizures were available for analysis. The origin was temporal in 68 seizures (15 patients), frontal in 39 seizures (three patients), parietal in four seizures (one patient) and occipital in eight seizures in one patient. In another patient localisation remained unclear (five seizures).

Temporal and frontal seizures were analysed. Because of the low number of seizures of occipital and parietal origin, these were excluded from analysis.

Temporal distribution of seizures correlated to the DLMO

The 68 seizures originating from the temporal lobe occurred significantly more frequently in the six hours before DLMO (DLMO bin II, p=0.001, see figure 2A). Fewer seizures than expected were seen in the six hours after DLMO (DLMO bin III, p=0.006).

The 39 seizures originating from the frontal lobe were seen most frequently six to twelve hours after DLMO (DLMO bin IV, see figure 2B) and significantly fewer seizures than expected were seen in the 12 hours before DLMO and six hours after DLMO (bin I, p=0.051, bin II, p=0.019 and bin III, p=0.003).

Temporal distribution of seizures over the 24-hour day

Significantly more temporal lobe seizures were observed in the time period 1100-1700h (p=0.021) and significantly fewer were seen during the night (2300-0500h, p=0.029). Frontal lobe seizures occurred most often from 2300-0500h (p<0.001) and fewest were observed from 1700 to 2300h (p=0.019).

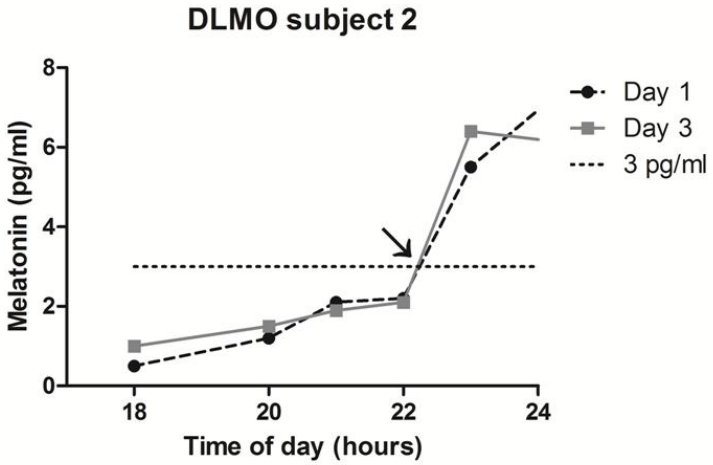

Figure 1. Diagram illustrating the saliva melatonin profile in subject 2 over the first 6 hours. The dim light melatonin (DLMO) onset is indicated with an arrow.

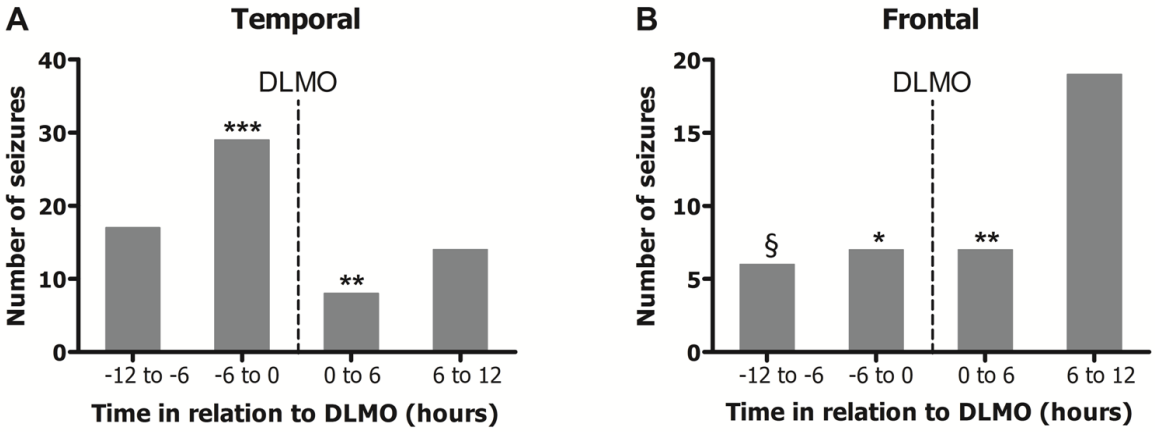

Figure 2. Bar graphs showing the temporal distribution of seizures from temporal (A) and frontal origin (B) as correlated to the circadian phase (DLMO=dim light melatonin onset). Bars with * p<0.05,** p<0.01, *** p=0.001, or § p=0.051 deviate significantly from a uniform distribution.

Discussion

The main findings of this study were that seizures of temporal origin and of frontal origin seem to be time locked to the circadian phase of the patient. Furthermore, non-random seizure patterns from temporal and frontal origin over the 24-h day were confirmed.

In animal studies clear 24-h seizure patterns in various epilepsy models have been observed. For example, in studies of rodents with limbic epilepsy it was observed that seizure latency was shorter and more spontaneous seizures occurred during exposure to light than during darkness (Bertram and Cornett, 1994; Cavalheiro et al., 1991; Gorter et al., 2001; Hellier and Dudek, 1999; Quigg et al., 1998b; Quigg et al., 2000). One study proved that spontaneous limbic seizures in rats occur in a true endogenously mediated circadian pattern (Quigg et al., 2000).

Likewise, several studies in humans have shown 24-h rhythmicity in seizure occurrence. In one study, seizure patterns of patients with mesial temporal lobe epilepsy (MTLE), extraTLE (XTLE) and lesional TLE (LTLE) were compared to the occurrence of seizures in a rat model of limbic epilepsy (Quigg et al., 1998b). It was found that seizures in LTLE and XTLE occur randomly, but seizures in MTLE occurred in a daily cosinor distribution with a peak incidence at approximately 1500h. This was comparable to the distribution in time of seizures observed in the rats, thus in relation with the light-dark cycle. In another study, Pavlova et al. found a significant peak in seizure occurrence between 1500 and 1900h in patients with TLE and a peak between 1900 and 2300h in patients with XTLE (Pavlova et al., 2004). In our own epilepsy centre, we have evaluated temporal distribution of clinical seizures in adults and children (Hofstra et al., 2009a). Significantly more seizures were observed from 1100 to 1700h and fewer seizures were seen from 2300 to 0500h. Daytime peak incidences were observed for complex partial seizures, seizures of extratemporal origin (in children) and seizures of temporal origin (in adults). Significantly fewer seizures of temporal and extratemporal origin (in children) were observed from 2300-0500h. Two studies have been performed in which intracranial EEG monitoring (considered the gold standard) was used. In one study, non-uniform distributions were observed in seizures from the parietal, occipital, mesial temporal and neocortical temporal lobes (Durazzo et al., 2008). Occipital seizures peaked between 1600 and 1900h; parietal and frontal lobe seizures peaked between 0400 and 0700h. Two peaks were found in the occurrence of seizures in MTLE (1600-1900h and 0700-1000h). Seizures from the neocortical temporal lobe also peaked between 1600 and 1900h. In a recent study, we have analysed 450 spontaneous seizures in 33 patients with long-term intracranial EEG and video monitoring (Hofstra et al., 2009b). Seizures showed an uneven distribution over the day, depending on lobe of origin: temporal lobe seizures occurred preferentially between 1100 and 1700h, frontal seizures between 2300 and 0500h and parietal seizures between 1700 and 2300h.

All these studies showed diurnal rhythmicity, but unfortunately have not taken circadian rhythmicity into account. Therefore, it cannot be stated that the distribution over 24 hours is really circadian rhythmicity.

To date, only one study has focused on circadian rhythmicity, albeit not in seizures, but in interictal discharges (IEDs) (Pavlova et al., 2009). In this study three patients with generalized epilepsy underwent a forced desynchrony protocol under constant conditions. Subjects were placed in a time-isolated and dim lit room under constant environmental conditions. In this room subjects underwent an artificial short day length protocol, in which

they were allowed to sleep for 2 hours and 40 minutes and then were allowed to stay awake for the same period of time. In three days 11 recurring periods were scheduled. In this way circadian rhythmicity in IEDs could be studied without sleep pressure building up. It was found that there was an apparent circadian variation in IEDs with different phases relative to peak melatonin. In this interesting pilot study it was shown that the protocol used is feasible at least in patients with generalized epilepsy. However, subjects in this study were seizure free during the protocol. When having seizures or studying seizure patterns, this protocol might be less appropriate.

To our knowledge, ours is the first study focusing on seizure occurrence related to circadian phase. By employing the DLMO, which is the most robust phase marker, we were able to determine the circadian phase reliably. The collection of melatonin was performed under strict conditions, to minimize factors that are known to influence melatonin production.

Several factors limit conclusions in this study. The extent of influence of endogenous and exogenous factors on seizures and seizure timing is not known. Several factors have been described that can mask the true circadian rhythm (for review, see Hofstra and de Weerd, 2008). As our patients were studied under clinical diagnostic conditions, we could not perform a strict constant routine as is possible in a laboratory setting. However, we aimed for as constant conditions as possible whilst collecting saliva samples. Therefore, during the daytime measuring periods subjects were placed in a dimly lit living room with near constant temperature. They remained in an upright position, activity was minimized and certain food and drinks were prohibited. Meal times were kept constant throughout admission. During the night patients were in bed. Thus the last two saliva samples have been obtained after a change of posture from upright to supine. As the DLMO is likely to occur in the hours before habitual bedtime, the change of posture is not likely to have changed our results. Furthermore, subjects abstained from smoking, as it has been shown that this can influence the level of melatonin (Gordijn et al., 1991).

Further research will be needed to strengthen the hypothesis that circadian rhythmicity and epilepsy interact. Ideally, subjects with epilepsy should be measured under total constant conditions as Pavlova and others did. Further research will be needed to prove whether this can be done appropriately. If this is possible, other aspects of the hypothesized relationship between epilepsy and circadian rhythmicity could be studied, for instance, the effect of seizures on circadian mediated rhythms and vice versa, such as melatonin. Meanwhile, research on animal epileptic models under constant conditions could provide us with answers that cannot be obtained (yet) with human studies. Furthermore, previous studies showed that patterns of temporal seizures are alike in rats and humans. The activity of the SCN and the melatonin production are two of the rare rhythms occurring in phase in these species, which can offer leads to elucidate the underlying mechanisms causing the specific seizure patterns.

In conclusion, this pilot study has demonstrated that seizures from temporal origin and frontal origin seem to be time locked to the individual circadian phase. This strongly suggests that circadian rhythmicity and seizures do indeed interact.

Chapter Nine

Summary and general discussion

Summary

In this thesis we investigated the influence of circadian rhythms on epilepsy. Circadian rhythms are endogenously mediated ~24-h cycles of physiological and psychological processes, including the sleep-wake cycle, core body temperature, blood pressure, task performance and hormone production. These circadian rhythms in mammals are generated and maintained by a biological clock in which the master circadian pacemaker is formed by the cells of suprachiasmatic nuclei (SCN). In addition to the master pacemaker in the SCN, there is convincing evidence for the existence of peripheral circadian oscillators in the human body. More or less independent peripheral oscillators are found in several organs, including the liver, skeletal muscle and testis; all are under the influence of the SCN (Lamont et al., 2007). To synchronize the circadian system to the 24-h day, the SCN need to adjust daily. This is termed entrainment and this is accomplished by external cues, so-called Zeitgebers ("time givers" in German), such as scheduled sleep, activity, temperature and by far the most important the solar light-dark cycle (Duffy and Wright, Jr., 2005).

Several genes have been discovered that are at least partly responsible for this characteristic activity of the individual SCN and the interindividual differences. The activity depends on the expression of auto regulatory translation-transcription feedback loops of genes including the *Period* genes (*Per1, Per2, Per3*), the *Clock* gene and two *Cryptochrome* genes (*Cry1, Cry2*). It has been demonstrated in several animal studies that deletion or mutation of these genes leads to rhythms with abnormal periods or even arrhythmic phenotypes when tested under constant conditions. Moreover, dysfunction of these clock genes might be important in the development of various diseases, including cancer (Lamont et al., 2007).

In **Chapter 2** the relatively poor knowledge on the interaction between circadian rhythms and human epilepsy is discussed. If this relationship exists, this interaction may be of value for better knowledge of pathophysiology and for timing of diagnostic procedures and therapy, as therapy adjusted to individual circadian rhythmicity (an example of chronotherapy) might improve seizure control. It appears that human seizure occurrence may have 24-h rhythmicity, depending on the origin. These findings are supported by animal studies. Rats placed in constant darkness showed spontaneous limbic seizures occurring in an endogenously mediated circadian pattern. More studies are available on the influence of epilepsy on circadian rhythms. One group studied chronotypes in patients with different epilepsy syndromes and found significant differences in the distribution of chronotypes between these two groups. Numerous studies have described influences of epilepsy and seizures on sleep and vice versa. In contrast, knowledge on circadian (core) body temperature patients is minimal as is the knowledge on clock genes in patients. Reduced heart rate variability and changed hormone levels, which are under the influence of the biological clock, have been observed in people with epilepsy. In short, large gaps in the knowledge about the interaction of circadian rhythm and human epilepsy still remain.

In **Chapter 3** the methodology of measuring the circadian rhythm in humans is explored. An overview of widely used methods includes protocols used to desynchronize circadian rhythm and sleep-wake, such as the forced desynchrony protocol (i.e. living on a 20 or 28-h day), constant routine protocol (in which factors influencing circadian rhythmicity are minimized or

kept as constant as possible). Also, biological markers are employed to determine the phase of the circadian rhythm. Examples are the dim light melatonin onset (DLMO, i.e. the time the melatonin level starts rising in the evening under dim light conditions), core body temperature and cortisol. Sleep parameters are being used frequently, but fall short in comparison to the other reviewed methods. Questionnaires are helpful in determining chronotypes and sleep parameters and finally, actimetry is one of the most frequently used methods in animal circadian studies, but in human studies merely a good additional tool. In conclusion, the DLMO is the most robust and most widely employed method to measure circadian rhythmicity in humans.

Very few studies have evaluated seizure occurrence in humans over the 24-h day; data from children are particularly scarce. In the study described in **Chapter 4** we have analysed clinical seizures of 176 consecutive patients (76 children, 100 adults) who had continuous electroencephalography (EEG) and video monitoring lasting more than 22 hours. Several aspects of seizures were noted, including classification, time of day, origin and sleep stage and seizure numbers were compared to numbers expected when seizures would occur randomly (binomial test). More than 800 seizures were recorded. Significantly more seizures than expected when occurring randomly were observed from 1100 to 1700h and from 2300 to 0500h significantly fewer seizures than expected were seen. The daytime peak incidences were observed in all types of seizures, but also in subgroups with complex partial seizures (in children and adults), seizures of extratemporal origin (in children) and seizures of temporal origin (in adults). Incidences significantly lower than expected were seen in the period 2300 to 0500h in all types of seizures, complex partial seizures (in children and adults) and in tonic seizures (in children). In addition, significantly fewer seizures of temporal (in children and adults) and extratemporal origin (in children) were observed in this period. The results suggest that certain types of seizures have a strong tendency to occur in true diurnal patterns. These patterns are characterized by a peak during midday and a minimum in the early night.

As mentioned above, few studies have evaluated human seizure occurrence over the 24-h day and only one group has employed intracranial electrocorticography monitoring to record seizures. We have analysed spontaneous seizures in 33 consecutive patients with long-term intracranial EEG and video monitoring. This study is described in **Chapter 5**. Several aspects of seizures were noted, including time of day, origin, type and behavioural state (sleeping/awake). We recorded 450 seizures that showed an uneven distribution over the day, depending on lobe of origin: temporal lobe seizures occurred preferentially between 1100 and 1700h, frontal seizures between 2300 and 0500h and parietal seizures between 1700 and 2300h. In the awake state, larger proportions of clinical seizures were seen from 0500 to 1100h and from 1700 to 2300h. During sleep, larger proportions occurred from 1100 to 1700h and from 2300 to 0500h. Our results suggest that seizures from different brain regions have a strong tendency to occur in different diurnal patterns.

It is conceivable that seizure timing could influence timing of daily activities, sleep and wake (i.e. chronotype). Therefore, we performed a questionnaire study to compare the distribution of chronotypes and sleep parameters in 200 epilepsy patients to the distributions in the general population. This study is described in **Chapter 6**. To determine chronotypes and

subjective sleep parameters the Morningness Eveningness Questionnaire and the Munich Chronotype Questionnaire were used. Significant differences were found between people with epilepsy and healthy controls. Epilepsy patients were more morning oriented, had an earlier mid sleep on free days and sleep duration on free days was longer (p<0.001). However, the distribution of chronotypes and subjective sleep parameters between patients with temporal lobe epilepsy, frontal lobe epilepsy and juvenile myoclonic epilepsy was found not to be different. Also, patients that had been operated on temporal lobe epilepsy had similar chronotypes and sleep duration when compared to patients who were not operated, but mid sleep on free days was earlier (p=0.035). In conclusion, this is the first large study focusing on chronotypes in epilepsy patients. We show that the distribution of chronotypes and subjective sleep parameters in patients in general is different from that of controls. Nevertheless, no difference is observed between patients with specified epilepsy syndromes, although they exhibit seizures in different diurnal seizure patterns. Our results suggest that epilepsy in general, but not seizure timing has significant influence on the chronotypes and sleep parameters.

Almost one-third of epilepsy patients continue to have seizures despite adequate drug treatment. Chronotherapy (based on dynamic changes in drug pharmacology and disease-related processes) could be a promising new treatment option. In the study described in **chapter 7**, we aimed to explore whether different circadian types (i.e. morning, intermediate and evening types) already adjust administration times of their anti-epileptic drugs (AEDs) as a first step in exploring chronotherapeutic possibilities. Therefore, we performed a questionnaire based study to compare the behaviour of patients with different circadian types in relation to the times of taking drugs. Circadian type (morning, intermediate or evening type) was determined by the Morningness/Eveningness Questionnaire. Results clearly show that morning types are taking their AEDs significantly earlier than evening types do on free days, that is 100 minutes earlier for the morning dose (p<0.001) and 55 minutes earlier for the evening dose (p=0.019). Also, times of taking AEDs in the morning on work days differ significantly between morning and evening types (55 minutes, p<0.001). Regardless of circadian type, drugs on free days are taken significantly later than on working days, which is most pronounced in evening types (up to 90 minutes delay in the evening types, p=0.005). Age and gender did not influence times of taking AEDs. In conclusion, this is the first study to show that patients adapt times of taking medication to their circadian type.

As mentioned, there is strong evidence that epileptic seizures occur in diurnal patterns. A study in rat models of partial epilepsy showed circadian seizure patterns and in humans circadian rhythmicity in interictal discharges has been found, suggesting that circadian rhythm may play a role in epilepsy. Circadian influences on human seizure patterns have not been investigated. In **chapter 8** the study is described in which we performed a pilot study to ascertain influences of the circadian rhythm on seizure occurrence. We prospectively outlined circadian rhythms of patients admitted for long term EEG-video monitoring, using measurement of the dim light melatonin onset (DLMO). Seizures during admission were recorded with continuous EEG and video monitoring. The DLMO ranged from 1846h to 2313h (mean 2122h). One hundred and twenty-four seizures of 21 patients were analysed. Seizures of temporal lobe origin occurred mainly between 1100 and 1700h and frontal seizures were seen mostly between 2300 and 0500h. When correlating seizure timing to the

individual circadian phase as measured by the DLMO, the following was seen: temporal seizures occurred most frequently in the six hours before DLMO and frontal seizures mainly in six to twelve hours after the DLMO. The results of this pilot study suggest that temporal and frontal seizures not only occur in diurnal patterns, but are time locked to the circadian phase.

Conclusions

Based on the studies presented in this thesis the following conclusions can be drawn:

- Seizures in epilepsy patients do not occur randomly over the 24-h day, but follow certain temporal patterns. These patterns depend on the origin and type of seizure. Temporal seizures occur most frequently during midday, frontal seizures are observed mainly at night and parietal seizures mostly in the evening. Complex partial seizures are seen most frequently during daytime.
- When referenced to the individual circadian phase, temporal seizures are mostly observed in the six hours preceding the dim light melatonin onset (a robust marker for the circadian phase) and frontal seizures occur most frequently in the six to twelve hours after the dim light melatonin onset.
- Epilepsy in general, but not seizure timing has significant influence on the chronotypes and subjective sleep parameters. People with epilepsy are more morning oriented and have an earlier mid sleep on free days than people without epilepsy.
- In our patient population we have shown that people adapt the times they take their drugs to their level of morningness or eveningness. Also, times of taking medication are delayed significantly on free days as compared to work days.

Overall, the results of our study strongly suggest that there is interaction between epilepsy and circadian rhythmicity.

Discussion and Future Perspectives

Over a century ago, Gowers classified seizure occurrence as diurnal, nocturnal and diffuse (Gowers, 1885). In animal studies with models of temporal epilepsy clear diurnal patterns of epileptic seizures have been found, with more spontaneous seizures during the light phase than during darkness (Halberg & Howard, 1958; Freeman, 1980; Cavalheiro et al., 1991; Bertram & Cornett, 1994; Quigg et al., 1998; Hellier & Dudek, 1999; Quigg et al., 2000; Gorter et al., 2001; Torshin & Vlasova, 2001). Also in humans, this field is beginning to be explored and studies confirmed the non-random patterns first with seizure diaries and later on with scalp EEG recordings and even intracranial EEG monitoring (considered the gold standard) (Durazzo et al., 2008; Pavlova et al., 2004; Quigg et al., 1998b; Quigg and Straume, 2000). Our studies described in Chapter 4 and 5 expand the limited human knowledge on human 24-h seizure rhythmicity measured by scalp and intracranial EEG (Hofstra et al., 2009a; Hofstra et al., 2009b). In one study, temporal seizure patterns were compared to the occurrence of seizures in a rat model of limbic epilepsy and it was found that the distribution of seizures over the 24-h day was similar in humans and rats (Quigg et al., 1998b).

The above mentioned studies showed 24-h, i.e. diurnal, rhythmicity in seizure occurrence, with similarities between human and animal seizure patterns. However, the

thought that this rhythmicity might be mediated by the circadian system and therefore being a circadian rhythm, as many other physiological and psychological processes in the body, is focus of far more recent research.

Up till now, however, only very few studies have focused on true circadian rhythmicity in seizures. In one study, it was shown that spontaneous limbic seizures in rats occur in a true endogenously mediated circadian pattern (Quigg et al., 2000). Another study has focused on circadian rhythmicity in interictal discharges (IEDs) in three patients and found true circadian rhythmicity in IEDs (Pavlova et al., 2009). Our study as discussed in chapter 8 is the only one that focuses on the time relation between human seizure occurrence and circadian rhythmicity. We observed how seizures from temporal and frontal origin seem time locked to the individual circadian phase.

The key question is why seizures occur in diurnal and probably circadian patterns. We know that temporal seizures in humans and rats occur in-phase, although humans are diurnal and rats nocturnal. There are only three rhythms in-phase, which are the activity of the SCN, the vasopressin rhythm and the melatonin rhythm.

The knowledge on the role of vasopressin (VP) with respect to epilepsy and seizures is limited. Vasopressin, also known as argenine vasopressin, antidiuretic hormone or angiopressin is a peptide hormone that plays a key role in water balance. Vasopressin has been shown to rapidly increase during the phase of generalisation of a seizure, with the level peaking in the postictal phase and remaining high for several hours (Meierkord et al., 1994). The hormone has been thought to be proconvulsant, as rats that lack VP genetically require higher temperatures to induce heat-induced convulsions (Kasting et al., 1981). When tested in a febrile convulsion model in rat pups and a temporal lobe epilepsy model in adult rats, it was suggested that VP has a convulsant activity in febrile convulsions and also in seizures independent of fever (Gulec and Noyan, 2002). However, it has also been shown that the administration of VP to genetically epileptic gerbils reduces seizure occurrence (Lee and Lomax, 1983). More research is needed to interpret the precise role of VP in respect to epilepsy and seizures, to determine whether this hormone may play a key role in the interaction of circadian rhythmicity and epilepsy. A final answer on its role cannot be given because of the marginal recent knowledge.

Melatonin, on the other hand, has been studied intensively with respect to epilepsy and seizures. In several animal studies it has been shown that melatonin has anticonvulsant effects against electrically-induced seizures (Lapin et al., 1998; Mevissen and Ebert, 1998). Also, removing the pineal gland leads to seizure activity, which can be counteracted by the administration of exogenous melatonin (Rudeen et al., 1980). In humans the effect of epilepsy on melatonin and vice versa has been described in several studies. Results, however, are conflicting. Some authors describe low baseline levels of melatonin in people with epilepsy (Bazil et al., 2000; Fauteck et al., 1999; Laakso et al., 1993; Yalyn et al., 2006), whilst others found elevated levels (Molina-Carballo et al., 1994; Schapel et al., 1995). Likewise, elevated levels during or directly after complex partial seizures (CPS) and generalized tonic clonic seizures (GTCS) have been described (Bazil et al., 2000; Molina-Carballo et al., 2007), whilst other authors observed no changes after CPS and GTCS (Rao et al., 1989).

The effect of melatonin on seizure frequency has also been studied. It has been found that administration of melatonin to people with epilepsy can prevent seizures (Fauteck et al.,

1999; Molina-Carballo et al., 1997; Peled et al., 2001). With discontinuation of melatonin, seizure rates increased to previous levels (Molina-Carballo et al., 1997). In other studies, however, it was found that melatonin had no clear group effect on seizure frequency or was even associated with increased seizure activity in children with generalized epilepsy (Coppola et al., 2004; Sheldon, 1998). The precise role of melatonin in epilepsy and seizure occurrence is so far thus unclear. In my opinion, it is likely that melatonin at least interacts in the spectrum of facilitating and inhibiting factors of seizure occurrence. However, it might be that its role differs in different types of seizures and maybe also in different circadian phases. In order to find the answer, thorough studies, preferably randomized controlled trials are needed.

Another factor that might determine the circadian patterns of seizures is the chronotype. This term refers to the preferred phase of an individual for the timing of daily activities, sleep and wakefulness. So-called morning types (“larks”) tend to adopt an earlier sleep schedule than evening types (“owls”) and peaks in performance and alertness are seen earlier in the day in morning than in evening types (Horne and Ostberg, 1976; Kerkhof and Van Dongen, 1996; Roenneberg et al., 2004; Roenneberg et al., 2007). These individual preferences for sleep and wake have hardly been studied in relation to epilepsy. In a few studies of rodent epilepsy models daily activity patterns were studied. However, these studies do not agree on whether seizures or electroshocks result in a phase shift in behavioural rhythms and in what direction the amplitude is altered and are thus not helpful to answer this question (Angles-Pujolras et al., 2009; Smith et al., 2009; Stewart et al., 2006; Stewart and Leung, 2003). Results are even fewer in humans. More than half a century ago Janz and Christian described that patients with JME have the tendency to get up late in the morning, are most active in the afternoon and evening and fall asleep late at night (Janz and Christian, 1957). Decades later Pung and Schmitz (2006) compared 20 JME patients and 20 patients with TLE and found significant differences between the circadian activity patterns; patients with JME tended to go to bed later at night, to get up later in the morning and to feel fit at a later time during the day than patients with TLE. In our study including 200 epilepsy patients versus 4042 controls we found no differences in chronotype or sleep parameters between patients with three different epilepsy syndromes, being JME, TLE and FLE. Results however did show that people with epilepsy in general are more morning oriented and sleep longer on free days. The reason for the stronger morning orientation has yet to be elucidated. Sleep of epilepsy patients overall is of poorer quality than that of controls, as in many other (neurological) diseases. This could be part of the explanation for the longer total sleep time on free days, as patients try to compensate.

Many epilepsy patients continue to have seizures with the current drugs. Amongst others, chronotherapy may offer a solution to drug-resistant epilepsy. A first step would be to make an inventory of the times drugs are taken by patients in practice. To our knowledge, this has not been published before. Ours is the first study focusing on the influence of circadian typology in drug administration times. We found that epilepsy patients adapt their drug administration times to their circadian type. On free days, morning types take their AEDs significantly earlier than evening types do, with most pronounced differences in the morning doses. Also, times of taking AEDs in the morning on work days differ between morning and evening types. Furthermore, in general, drug administration times are significantly delayed

on free days compared to work days. As results of this study show that patients take their AEDs adapted to their level of morningness/eveningness, physicians need to realize this when prescribing drugs to their patients. Such irregularity could contribute to poor seizure control. In case of poor seizure control, it may be helpful to ask patients to fill in their drug administration times on work days and free days and to emphasize the importance of taking drugs at the same time every day, regardless of whether it is a free day or work day.

Methodological considerations

The extent of influence of endogenous and exogenous factors on seizures and seizure timing is not known. Several factors have been described that can mask the true circadian rhythm, such as the light-dark cycle, the sleep-wake cycle, physical exercise, posture, meal times and so on (for review, see (Hofstra and De Weerd, 2008)). Furthermore, because of this light-dark cycle and other so-called entraining factors, the circadian system of our patients is synchronized to the outside day. This means, that to measure circadian rhythm and circadian influences in a more pure way certain protocols are needed, such as a forced desynchrony or constant routine protocol. In our studies we could not apply such protocols. It is also questionable whether such protocols can be applied safely in epilepsy patients, because of sleep deprivation. Furthermore, it is ethically challenging to perform such protocols in patients. In the study described in chapter 8 we have applied a semi-constant routine protocol, in which important masking factors in the measurement of the DLMO were kept as minimal as possible.

Another exogenous factor that has to be taken into account with interpretation of the results is the use of drugs. The great majority of our subjects use anti-epileptic drugs. Even more complicating is the fact that many patients use more than one type of drug and that during long-term admission drugs are often tapered to increase the probability of having seizures. Even under steady state conditions, fluctuations in drug levels occur. This could lead to differential inhibition of seizures over the 24-h day. However, effects of AEDs on the distribution of seizures remain to be elucidated, as reports disagree (Griffiths and Fox, 1938; Helmchen et al., 1964). Not only the influences of AEDs on seizure patterns is of importance. Also the effects of drugs on other circadian rhythms have to be accounted for. For example, it has been shown that VPA lengthens circadian locomotor rhythm in Drosophila (Dokucu et al., 2005), however, no effect on the wheel-running circadian rhythm in golden hamsters was shown (Klemfuss and Kripke, 1995). The studies performed were all based on a patient population in a tertiary epilepsy clinic. Our database does therefore not represent the average population of epilepsy patients. For instance, complex partial seizures contribute disproportionally and primary generalized seizures are not represented at all.

Finally, some observations in our studies will be strengthened if measured in other ways. For instance, the chronotype study and the study with timing of AEDs is performed with questionnaires and is worth being endorsed by measuring for example the melatonin curve, to see whether the endogenous rhythms are similarly characteristic.

Clinical implications

Knowing that seizures from different types and origins occur in different patterns implies that diagnostic yield might be improved by adjusting timing of diagnostic options (such as EEG monitoring) to the expected type and/or origin of seizures. For instance, short-term EEGs to capture temporal seizures can preferably be scheduled at midday or in the afternoon, as our

results strongly support that these seizures tend to occur more frequently in that period. The same goes for scheduling an EEG overnight to capture frontal seizures.

Furthermore, in various diseases chronotherapy has proven its success. In epilepsy, adjustment of therapy to individual circadian patterns might very well improve effects of AEDs and thereby improve seizure control and decrease adverse effects, although at the moment more research is needed to confirm this hypothesis.

Further research

With the studies described in this thesis we have clearly strengthened the hypothesis that circadian rhythmicity and epilepsy interact. However, the underlying mechanisms remain to be elucidated. For instance, what causes rhythmicity in seizures or the fact that epilepsy patients are more morning oriented and have an earlier mid sleep on free days? As mentioned above, the SCN activity, the melatonin and vasopressin rhythms are the only rhythms to occur in-phase in nocturnal and diurnal animals. Therefore, it is very likely we will find an answer in why seizure rhythms in humans and rats are also in-phase by studying these rhythms in further detail. Also, because it has already been shown that seizures in a rat model for temporal lobe epilepsy occur in a circadian rhythm. The difficulty lies in the broad spectrum of endogenous and exogenous factors that all interact in epilepsy and seizure occurrence. More studies are needed to fill in these gaps in the knowledge about the interaction of circadian rhythm and epilepsy. Ideally, subjects with epilepsy should be measured under totally constant conditions as Pavlova and others did (2009). In this interesting pilot study it was shown that the forced desynchrony protocol used is feasible at least in patients with generalized epilepsy. However, subjects in this study were seizure free during the protocol. When having seizures or studying seizure patterns, this protocol might be less safe to use. Further research will need to prove whether this can be done safely. As we have shown, delineating circadian phase by means of the DLMO is a perfectly safe way of studying circadian rhythmicity in epilepsy patients, even though seizures occur frequently.

With constant routine or forced desynchrony protocols or by delineation of the circadian phase, the hypothesized relationship between epilepsy and circadian rhythmicity can be studied in more detail. Meanwhile, research on animal epileptic models under constant conditions could provide us with answers that cannot be obtained (yet) with human studies. Future studies could focus on, for instance, the effect of seizures on circadian mediated rhythms such as melatonin. Another interesting option would be to phase shift the rhythm in epileptic animal models, to see whether seizure rhythms follow. Also, further research is needed to explore the underlying mechanisms of circadian patterns in seizure occurrence, to elucidate what causes the different seizure patterns. Furthermore, the effects of anti-epileptic drug treatment adjusted to the circadian rhythm could be studied, to see whether adaptation of treatment to the individual circadian rhythm would improve seizure control.

Therefore, the results from the studies described in this thesis strengthen the believe that circadian rhythmicity and epilepsy closely interact. With numerous questions answered and other questions risen, further studies are needed in this relatively new field of epilepsy research, to further explore pathophysiology, diagnostic and most important therapeutic options in epilepsy.

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Nederlandse samenvatting

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Circadiane Ritmiek en Epilepsie: het Belang van Chronobiologische Tijd

In de studies beschreven in dit proefschrift hebben we de interactie tussen circadiane ritmiek en epilepsie onderzocht. Circadiane ritmen zijn endogeen bepaalde cycli van ongeveer 24 uur. Deze cycli worden in veel fysiologische en psychologische ritmen gevonden, zoals slaap-waak ritme, kernlichaamstemperatuur, bloeddruk, het uitvoeren van taken en hormoonproductie. Circadiane ritmen worden in zoogdieren gegenereerd en onderhouden door een biologische klok waarvan het hoofdbestanddeel wordt gevormd door cellen in de suprachiasmatische kernen of nuclei (SCN). Naast de SCN worden er ook perifere circadiane oscillatoren in het menselijk lichaam gevonden, die min of meer onafhankelijk van de SCN werken. Deze oscillatoren zijn onder andere aangetoond in de lever, skeletspieren en testis en staan allemaal onder invloed van de SCN (Lamont et al., 2007). Om het circadiane systeem gelijk te laten lopen aan de 24-uurs dag, moeten de SCN zich elke dag aanpassen of 'resetten'. Dit wordt 'entrainment' genoemd en wordt bereikt door externe aanwijzingen, die ook wel Zeitgebers ("tijdgevers") worden genoemd. Zeitgebers zijn bijvoorbeeld geplande slaap, activiteiten en temperatuur. Verreweg de belangrijkste Zeitgeber is de licht-donkercyclus door de zon (Duffy and Wright, Jr., 2005).

Er zijn diverse genen ontdekt die, tenminste deels, verantwoordelijk zijn voor de karakteristieke activiteit van de individuele SCN en de verschillen tussen individuen. De activiteit hangt af van de expressie van zelf-regulatoire translatie-transcriptie feedback loops van genen zoals de *Period* genes (*Per1, Per2, Per3*), het *Clock* gen en twee *Cryptochrome* genen (*Cry1, Cry2*). In verschillende dierstudies is aangetoond dat deletie of mutatie in deze genen tot ritmen met abnormale perioden leidt of zelfs tot aritmische fenotypen, als je de diermodellen onder constante condities test. Bovendien wordt gedacht dat dysfunctie van deze genen een belangrijke rol speelt in de ontwikkeling van verschillende ziekten, waaronder kanker (Lamont et al., 2007).

De onderzoeken in dit proefschrift hebben zich gericht op de interactie tussen circadiane ritmiek en epilepsie. Epilepsie is een veelvoorkomende neurologische aandoening. Bij een epileptische aanval raakt het normale activatiepatroon van neuronen verstoord. Hierdoor kunnen aanvallen ontstaan met vreemde sensaties, emoties, spierverkramping en/ of verlaging van het bewustzijn. Volgens de Wereld Gezondheids Organisatie (World Health Organisation, WHO) is epilepsie de meest voorkomende ernstige hersenaandoening wereldwijd met vandaag de dag 40 tot 50 miljoen patiënten. Geschat wordt dat er wereldwijd twee miljoen nieuwe gevallen per jaar bijkomen. In Nederland wordt het aantal epilepsiepatiënten geschat op 121000, dit in vergelijking met bijvoorbeeld 16000 patiënten met multipele sclerose en 26000 patiënten met de ziekte van Parkinson (cijfers uit 2008 (Gommer and Poos, 2010a, b en c)).

In **hoofdstuk 2** wordt de bestaande kennis over de interactie tussen circadiane ritmiek en epilepsie bij mensen uiteen gezet. Als er inderdaad een relatie is, kan de interactie van belang zijn voor een beter begrip van de pathofysiologie van epilepsie en ook voor het tijdstip waarop diagnostiek en behandeling plaatsvinden. Het zou bijvoorbeeld zo kunnen zijn dat het aanpassen van de behandeling aan het individuele circadiane ritme (een voorbeeld van

chronotherapie) de controle over aanvallen kan verbeteren. Het lijkt erop dat het voorkomen van epileptische aanvallen bij de mens een 24-uurs ritme volgt, afhankelijk van uit welke hersenkwab de aanval komt. Deze bevindingen worden ondersteund door resultaten van onderzoeken in dieren. Ratten die in continue duisternis geplaatst werden, lieten spontane limbische aanvallen zien, die in een endogeen bepaald circadiaan ritme voorkwamen.

Daarnaast zijn er verschillende studies verricht naar de invloed van epilepsie op circadiane ritmen. Significante verschillen in chronotypen werden gevonden tussen twee groepen patiënten met verschillende epilepsiesyndromen. Talloze studies beschrijven de invloed van epilepsie en epileptische aanvallen op de slaap en vice versa. De kennis over kernlichaamstemperatuur en klokgenen in patiënten is echter (nog) minimaal. Wel is er in diverse studies gevonden dat er een verminderde variabiliteit in hartritme (een circadiaan ritme) bestaat in epilepsiepatiënten. Ook kunnen de spiegels van hormonen, die onder invloed staan van de 24-uurs biologische klok, anders zijn bij epilepsiepatiënten.

Kortom, er zijn nog steeds grote hiaten in de kennis over de interactie tussen circadiane ritmiek en epilepsie.

In **hoofdstuk 3** wordt beschreven hoe je circadiane ritmiek kunt meten bij mensen. Er wordt een overzicht gegeven van vaak gebruikte meetmethoden. Zo zijn er bepaalde belangrijke protocollen nodig om circadiane ritmiek in kaart te brengen. Een voorbeeld is een desynchronisatieprotocol waarin circadiane ritmiek en de slaap-waak cyclus uiteen kunnen worden gehaald of een ‘constante routine’-protocol, waarin factoren die circadiane ritmiek kunnen beïnvloeden zo minimaal of constant mogelijk worden gehouden. Tevens worden biologische markers besproken, die de fase van het circadiane ritme kunnen bepalen. Voorbeelden hiervan zijn het moment dat de melatoninespiegel toeneemt (bij gedimd licht, in het Engels *dim light melatonin onset, DLMO* genoemd), de kernlichaamstemperatuur en de cortisolspiegel. Slaapparameters worden ook vaak gebruikt, maar schieten tekort in vergelijking met de hierboven beschreven methoden. Vragenlijsten zijn behulpzaam in het bepalen van chronotypen en slaapparameters. Verder is actigrafie een van de meest gebruikte methoden in dieronderzoek naar circadiane ritmiek. In de mens is het echter niet een kernmethode, maar meer een goede aanvulling. Concluderend is de DLMO de meest robuuste en meest gebruikte methode om het circadiane ritme in mensen in kaart te brengen.

Slechts weinig studies hebben het vóórkomen van epileptische aanvallen over de 24-uurs dag geëvalueerd. Met name gegevens over aanvallen bij kinderen zijn schaars. In de studie die in **hoofdstuk 4** beschreven wordt, hebben we klinische aanvallen bij 176 patiënten (76 kinderen, 100 volwassenen) geanalyseerd. Deze patiënten waren opgenomen voor continue electro-encefalografie- (EEG) en videoregistratie met een duur van tenminste 22 uur. Verscheidene kenmerken van de aanvallen werden meegenomen in het onderzoek: classificatie, tijdstip, origine en in welk slaapstadium de aanval voorkwam. Aantallen aanvallen werden vergeleken met aantallen die verwacht kunnen worden als aanvallen willekeurig zouden plaatsvinden (binomiale test). Er werden meer dan 800 aanvallen geregistreerd. Beduidend meer aanvallen werden geobserveerd tussen 11 en 17 uur en tussen 23 en 5 uur werden significant minder aanvallen gezien dan je zou verwachten als aanvallen willekeurig zouden voorkomen. Pieken in voorkomen midden op de dag (11-17u) werden gezien in alle aanvallen bijeengenomen, maar ook in subgroepen met complex

partiële aanvallen (in zowel kinderen als volwassen), aanvallen uit extratemporale gebieden (in kinderen) en aanvallen uit de temporaalkwabben (in volwassenen). Significant minder aanvallen dan verwacht werden geobserveerd tussen 23 en 5 uur bij alle aanvallen bijeengenomen, complex partiële aanvallen (zowel kinderen als volwassen) en bij tonische aanvallen in kinderen. Tevens werden veel minder aanvallen uit de temporaalkwab (in zowel kinderen als volwassen) en van extratemporale origine (bij kinderen) dan verwacht gezien in deze periode.

Met deze resultaten lijkt het erop dat verschillende typen aanvallen in een bepaald ritme over de dag voorkomen. Deze patronen worden gekarakteriseerd door een piek midden op de dag en een dal aan het begin van de nacht.

Zoals hierboven genoemd, zijn er weinig studies die zich hebben gericht op het patroon van vóórkomen van aanvallen over de 24-uurs dag. Slechts één onderzoeksgroep heeft hierbij gebruik gemaakt van intracraniële EEG-metingen om aanvallen te registreren. Wij hebben de spontane aanvallen van 33 patiënten geanalyseerd die voor langdurige meting kwamen met intracraniële EEG en video. Deze studie wordt beschreven in **hoofdstuk 5**. Verscheidene kenmerken van de aanvallen werden meegenomen in het onderzoek: classificatie, tijdstip, origine en of de aanval voorkwam in waak of slaap. Er werden 450 aanvallen geregistreerd die ongelijk verdeeld over de dag voorkwamen, afhankelijk van origine van de aanval: temporale aanvallen werden vooral tussen 11 en 17 uur gezien, frontale aanvallen het meest tussen 23 en 5 uur en pariëtale aanvallen vooral tussen 17 en 23 uur. Tijdens waak kwam het grootste gedeelte van de aanvallen voor tussen 5 en 11 uur en 17 tot 23 uur. Als de patiënt in slaap was, werd het grootste gedeelte van de aanvallen geobserveerd van 11 tot 17 uur en 23 tot 5 uur. Deze resultaten lijken er op te wijzen dat aanvallen uit verschillende hersenkwabben een sterke tendens hebben voor te komen in verschillende patronen over de dag.

Het is denkbaar dat het moment waarop epileptische aanvallen voorkomen, invloed heeft op wanneer men slaapt, wakker is, dagelijkse activiteiten plant en doet (oftewel het chronotype). Daarom hebben we een studie uitgevoerd met vragenlijsten die de verdeling van chronotypen en slaapparameters bij 200 patiënten met epilepsie vergelijkt met de verdeling in de algemene bevolking. Deze studie wordt beschreven in **hoofdstuk 6**. Om het chronotype en de subjectieve slaapparameters te bepalen werden de Morningness Eveningness Questionnaire en de Munich Chronotype Questionnaire gebruikt. Er werden significante verschillen gevonden tussen epilepsiepatiënten en controles. Epilepsiepatiënten waren meer ochtend georiënteerd, hadden een vroegere midslaap (=tijdstip tussen in slaap vallen en wakker worden) op vrije dagen en de slaapduur op vrije dagen was langer (p<0.001). De verdeling van chronotypen en subjectieve slaapparameters echter, was niet verschillend tussen patiënten met temporale epilepsie, frontale epilepsie of juveniele myoclonische epilepsie. Tevens hadden patiënten die geopereerd waren voor temporaal epilepsie dezelfde verdeling in chronotypen en slaapduur wanneer ze vergeleken werden met niet-geopereerde patiënten. Midslaap was echter eerder in geopereerde patiënten (p=0.035). Dit is de eerste studie die zich richt op chronotypen in epilepsiepatiënten. We hebben laten zien dat de verdeling van chronotypen en subjectieve slaapparameters verschillend is wanneer epilepsiepatiënten en controles vergeleken worden. Niettemin hebben we geen verschil geconstateerd tussen patiënten met verschillende

epilepsiesyndromen, ook al worden hierbij verschillende aanvalspatronen over de 24-uurs dag gezien. Onze resultaten suggereren dat epilepsie op zich, maar niet de tijdstippen waarop aanvallen voorkomen, invloed heeft op chronotypen en subjectieve slaapparameters.

Bijna een derde van de patiënten met epilepsie houdt aanvallen ondanks adequate behandeling met medicijnen. Chronotherapie (gebaseerd op dynamische verandering in de farmacologie van medicijnen en ziekte gerelateerde processen) zou een veelbelovende nieuwe optie kunnen zijn in de behandeling van epilepsie. In de studie die wordt beschreven in **hoofdstuk 7**, was het doel te bestuderen of verschillende circadiane types (oftewel ochtend- en avondtypes en tussenliggende types) de tijden dat ze medicatie innemen aanpassen aan hun circadiane type of niet. Deze studie werd uitgevoerd als een eerste stap in het exploreren van chronotherapeutische opties. Om dit te onderzoeken hebben we een vragenlijststudie verricht, die de tijden van inname van anti-epileptische medicatie vergelijkt tussen patiënten met verschillende circadiane types. Het circadiane type werd bepaald door de Morningness/Eveningness Questionnaire. Resultaten laten duidelijk zien dat ochtendtypes hun medicatie op vrije dagen significant eerder innemen dan avondtypen met een verschil van wel 100 minuten bij het nemen van de ochtenddosis (p<0.001) en 55 minuten bij de avonddosis (p=0.019). Ook op werkdagen worden verschillen gezien: zo nemen ochtendtypen de ochtenddosis significant eerder in dan avondtypes (55 minuten, p<0.001). Onafhankelijk van het circadiane type werd medicatie op vrije dagen beduidend later ingenomen dan op werkdagen. Dit verschil is het grootst in de groep avondtypen (verschil van 90 minuten, p=0.005). Leeftijd en geslacht hadden geen invloed op hoe laat medicatie werd ingenomen. Concluderend kunnen we zeggen dat dit de eerste studie is die laat zien dat patiënten de tijden waarop ze medicatie innemen aanpassen aan hun circadiane type.

Zoals genoemd is er sterk bewijs dat epileptische aanvallen in diurnale patronen voorkomen. Een studie in een rattenmodel van partiële epilepsie liet circadiane aanvalspatronen zien en in mensen is circadiane ritmiek in interictale ontladingen gevonden. Dit suggereert dat circadiane ritmiek mogelijk een rol speelt in epilepsie. Circadiane invloed op aanvalspatronen in de mens is nog nooit bestudeerd. In **hoofdstuk 8** wordt de pilot studie beschreven die we hebben verricht om vast te stellen of circadiane ritmiek aanvalspatronen beïnvloedt. We hebben circadiane ritmes van patiënten, die werden opgenomen voor EEG en videoregistratie, prospectief bepaald. Hierbij hebben we gebruik gemaakt van de melatoninespiegel (het moment dat de spiegel stijgt onder gedimd-lichtcondities (dim light melatonin onset, DLMO)). Aanvallen die optraden tijdens de opname werden geregistreerd door EEG en video. De DLMO varieerde van 18:46u tot 23:13u (gemiddeld 21:22u). Honderdvierentwintig aanvallen van 21 patiënten werden geanalyseerd. Aanvallen uit de temporaalkwab kwamen met name tussen 23 en 5 uur voor. Als het tijdstip van aanvallen gecorreleerd werd aan de individuele circadiane fase (zoals gemeten met de DLMO) werd het volgende gezien: temporale aanvallen kwamen het meest voor in de zes uur voor DLMO en frontale aanvallen met name tussen zes en twaalf uur na DLMO. De resultaten van deze pilot studie suggereren dat temporale en frontale aanvallen niet alleen in diurnale patronen voorkomen, maar verbonden lijken te zijn met de circadiane fase.

Dankwoord

Dankwoord

Een proefschrift komt nooit tot stand zonder de inzet van vele anderen naast de promovendus zelf. Bij dezen wil ik dan ook een ieder hartelijk bedanken die er op welke manier dan ook aan heeft bijgedragen.

Mijn dank geldt ook zeker voor de patiënten; zonder hun medewerking waren deze studies nooit tot stand gekomen.

Daarnaast wil ik in het bijzonder de volgende mensen bedanken:

Dr. A.W. de Weerd: Al, mijn co-promotor, mijn begeleider. Dank voor de enthousiaste en directe begeleiding. Dank voor alle antwoorden op de korte vraagjes tussendoor. Dank voor de snelle feedback.

Prof. Dr. C.J. Stam: Kees, mijn promotor, bedankt voor het goede overzicht en de optimistische aanmoedigingen. Bedankt voor het vertrouwen dat we een heel eind konden komen in korte tijd.

Prof. Dr. J.W. Sander: Ley, thank you for your endless optimism and furthermore for your critical but positive feedback on my manuscripts. Dank!

De laboranten, mijn collega's. Natuurlijk moet ik jullie specifiek bedanken voor aan onderzoek gerelateerde activiteiten zoals het uitdelen van de vragenlijsten (m.n. Agnes B!). Daarnaast wil ik jullie echter ook (en misschien vooral!) bedanken voor de gezelligheid en de goede sfeer op de werkvloer! En de eindeloze toevoer van café au lait…
Daarnaast ben ik ook de verpleging grote dank verschuldigd voor het geduld met 'watjes kauwen' en in het schemerduister zitten (en dan is het niet altijd gemakkelijk om wakker te blijven), dus bij deze, bedankt!

Prof. Dr. J. van der Palen: Job, mijn vraagbaak voor de statistiek! Altijd snel antwoord inclusief grondige en begrijpelijke uitleg. Ik had niet zonder gekund… mijn dank is groot!

Dr. M.C.M. Gordijn: Marijke, bedankt voor de steeds diepergaande introductie en feedback op onze chronobiologische plannen. Zonder je hulp en meedenken was het niet gelukt!

De neurologen van SEIN: Mireille, Charles, Hanna, Yvette, Evelien, José en Jacques. Jullie ben ik dank verschuldigd voor de medewerking, feedback en leerzame besprekingen. Hierdoor heb ik veel kennis buiten mijn onderzoeksveld om opgedaan.

Heit, mem, Janneke en Nynke. Ek jimme hearre wis yn dit tankwurd te stean. Ik wol jimme betankje foar de soargeleaze jeugd en alle kânsen dy't jimme my jûn hawwe om my te ûntwikkeljen. Tige tank foar jimme folsleine steun, leafde en ynteresse.

Niels, onderaan dit dankwoord wil ik ook jou bedanken, terwijl je natuurlijk eigenlijk helemaal bovenaan hoort! Lieve Niels, ik heb het je niet gemakkelijk gemaakt met mijn studie- en

beroepskeuze, met mijn ambities. We gingen samen vanuit Driebergen en Doorn naar Groningen, daarna ging ik alleen naar Stockholm en daarna voor twee jaar naar Enschede. Gelukkig daarna weer samen door naar Zwolle en nu weer terug in Enschede. Ik ben je erg dankbaar voor je onvoorwaardelijke liefde en steun die je mij hebt gegeven en geeft om dit voor mij werkelijkheid te maken. Ik hoop dat we nog heel lang samen verder mogen reizen door het leven!

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List of publications

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Curriculum Vitae

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Wytske Ætske Hofstra werd op 28 november 1982 te Bedum geboren. In 2001 behaalde zij het gymnasiumdiploma op het Revius Lyceum te Doorn. Aansluitend studeerde ze geneeskunde aan de Rijksuniversiteit Groningen en volgde de co-schappen in het Medisch Spectrum Twente te Enschede. In februari 2008 behaalde zij haar artsentitel. Voorafgaand aan de opleiding Neurologie werkte ze vanaf eind 2007 als arts-onderzoeker op het Epilepsie- en Slaapcentrum SEIN in Zwolle. Gedurende ruim twee jaar werd de basis voor dit proefschrift gelegd. In maart 2010 begon ze haar opleiding tot neuroloog in het Medisch Spectrum Twente in Enschede.

Wytske Ætske Hofstra was born on the 28th of November 1982 in Bedum, the Netherlands. In 2001 she graduated from the Revius Lyceum in Doorn. Subsequently she studied Medicine at the University of Groningen and did her internships at the Medical Spectrum Hospital in Enschede. In february 2008 she graduated as MD. Preceding her residency in Neurology, she did a PhD-project at the Epilepsy and Sleep Centre SEIN in Zwolle. The work of these two years resulted in this thesis. In March 2010 she started her residency in the Medical Spectrum Twente in Enschede.