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.