The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/99307

Please be advised that this information was generated on 2018-07-14 and may be subject to change.
Endogenous rhythm of absence epilepsy: Relationship with general motor activity and sleep—wake states

Magdalena K. Smyk\textsuperscript{a,\*}, Anton M.L. Coenen\textsuperscript{b}, Marian H. Lewandowski\textsuperscript{a}, Gilles van Luijtelaar\textsuperscript{b}

\textsuperscript{a} Department of Neurophysiology and Chronobiology, Chair of Animal Physiology, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Krakow, Poland

\textsuperscript{b} Donders Centre for Cognition, Radboud University Nijmegen, P.O. Box 9104, 6500 HE Nijmegen, The Netherlands

Received 7 May 2010; received in revised form 24 September 2010; accepted 16 November 2010
Available online 13 December 2010

KEYWORDS
Absence seizures; Spike-wave discharges; WAG/Rij rats; Circadian rhythmicity; Motor activity; Sleep—wake states

Summary
The rhythms of spontaneously occurring seizures (spike-wave discharges, SWD) and motor activity, as well as the relationship between SWD and sleep—wake states were investigated in the WAG/Rij rat model of absence epilepsy. In order to establish whether SWD are controlled by external (Zeitgebers) or by endogenous factors such as circadian influences or the state of vigilance, the study was performed in entrained and constant dim light conditions.

EEG and motor activity were recorded in the 12:12 light—dark cycle and in constant dim light conditions. Circadian rhythmicity was found both for motor activity and the occurrence of SWD in conditions of entrainment. In constant dim light conditions also circadian rhythms emerged, however, the change in circadian parameters was opposite for the rhythm of SWD and motor activity. SWD were preceded mostly by passive wakefulness and by slow-wave sleep in both experimental conditions.

It can be concluded that the rhythm of SWD seems to be generated and controlled by an endogenous mechanism distinct from that which controls the rhythm of motor activity. The relationship between SWD and sleep—wake states preceding their occurrences appeared to be unchanged, suggesting that the mechanism of generation of SWD is independent of the circadian timing system.

© 2010 Elsevier B.V. Open access under the Elsevier OA license.

Introduction
The majority of mammalian physiological and behavioural processes are controlled by a circadian timing system. The light—dark cycle, the most prominent synchronizer of biological rhythms, entrains a circadian master clock, the
hypothalamic suprachiasmatic nuclei (SCN), to a 24-h day (Reppert and Weaver, 2001). The SCN control other clocks through multiple neuronal connections and humoral factors, and these clocks express overt circadian rhythms (Buïjs and Kalsbeek, 2001; Morin and Allen, 2006). These various oscillators or rhythms show a phase-relationship under entrained conditions.

However, the SCN generates a rhythm with a period length different from 24 h in absence of time cues (Bünning, 1964; Herzog and Schwartz, 2002). Not only the period may change, but also parameters such as amplitude and mean as well as the relationships between rhythms and even uncoupling of distinct circadian rhythms may occur. This phenomenon is called “internal desynchronization” and has been reported in humans and animals (Wever, 1979; Aschoff and Wever, 1981; Erket, 2000; Aguzzi et al., 2006). Internal desynchronization is thought to deliver evidence supporting a “multiple oscillatory theory” of the mammalian circadian timing system.

Also pathological processes such as the timing of epileptic seizures show daily rhythmic fluctuations. Investigations initiated by Gowers (1885), who classified seizures as diurnal, nocturnal and diffuse, led to the conclusion that distinct circadian patterns of the occurrence of epileptic discharges are determined by the type of epilepsy syndrome, both in humans and in animal models (Gowers, 1885; Langdon-Down and Brain, 1929; Pavlova et al., 2004; Durazzo et al., 2008; Hofstra et al., 2009; Quigg et al., 1998). It seems that the sleep–wake or rest–activity cycle plays a major role among the different circadian factors that ultimately influence the timing of seizures (Halasz, 1991). Changes in neuronal excitability associated with these rhythms determine the susceptibility for seizures to occur at specific or preferred states of vigilance.

Idiopathic generalized epilepsy (IGE) with typical absences belongs to a group of epilepsy syndromes that is strongly affected by the sleep–wake and rest–activity cycle. Indeed a 24-h rhythm has been identified for the occurrence of absence seizures (Kelly et al., 1980). Absences are characterized by the presence of bilateral, synchronous, generalized spike-wave discharges (SWD) in the EEG against a normal background activity. The frequency of the discharges is about 3 Hz and differs slightly among particular syndromes (Panayiotopoulos, 1994; Duncan, 1997). Slow-wave sleep, quiet wakefulness (e.g. physical and mental inactivity associated with drowsiness), light slow-wave sleep and transitions, mainly awakenings from slow-wave sleep are the most favourable states for SWD to occur, both in humans and in animal models (Coenen et al., 1991; Drinkenburg et al., 1991; van Luijtelaar and Bikbaev, 2007). SWD hardly occur during the highly desynchronized EEG activity, such as active wakefulness and REM sleep (Dinner, 2002; Halasz et al., 2002).

Rats of the WAG/Rij strain are a well known, validated, genetic animal model of absence epilepsy sharing common behavioural, electroencephalographic and pharmacological characteristics with the human disorder (Coenen and van Luijtelaar, 2003; Depaulis and van Luijtelaar, 2006). The distribution of the 7–11 Hz SWD show a clear circadian variation (van Luijtelaar and Coenen, 1988; van Luijtelaar et al., 2001), with a maximum during the early hours of the dark phase and a minimum at the beginning of the light phase, when rats have the highest amount of deep slow-wave sleep. The latter state is not favorable for the occurrence of SWD (Drinkenburg et al., 1991). Therefore, it is likely that the circadian distribution of SWD is a direct consequence of the distribution of the vigilance states and the presence of Zeitgebers.

The purpose of the present study was to verify the putative endogenous character of the rhythm of SWD. Considering that the probability of occurrence of SWD is higher during states of physical inactivity, emphasizing a role of vigilance in the generation of absences, the relationship between sleep–wake states including motor activity and the occurrence of SWD was additionally investigated in order to verify whether the absence of the Zeitgeber light on and off interferes with the dependency observed under conditions of entrainment.

### Materials and methods

#### Animals

Seven adult (eleven months old), male WAG/Rij rats, born and raised in the laboratory of the Donders Centre for Cognition of the Radboud University Nijmegen, served as subjects. Animals were maintained on a 12:12 light–dark cycle (lights on at 6:00 am) with food and water available ad libitum. All experimental procedures were approved by the Animal Ethical Committee of the Radboud University Nijmegen.

#### Surgery

Rats were implanted with a standard three-channel electrode set (MS 333/1-A, Plastic One Inc., Roanoke, VA, USA) under isoflurane anaesthesia. Two active electrodes were put on the surface of primary motor and visual cortex. Coordinates of the electrodes (with skull surface flat and bregma zero–zero) were: AP +2.0, ML 3.5 and AP −6.0, ML 4.0 respectively. The reference electrode was put over the cerebellum. Animals were allowed to recovery for 14 days after surgery.

#### EEG and activity recordings

Each animal was individually placed in sound-attenuated, ventilated recording cage and connected with a computer-based data acquisition system (Dataq Instruments, Akron, OH, USA). Rats were connected to a swivel which allowed them to move freely in their cages. The EEG signals were amplified, band pass filtered between 1 and 100 Hz, and sampled at 200Hz. General motor activity was measured by a passive infrared sensor (PIR) mounted 40 cm above the bottom of the animal’s cage and connected to the data acquisition system. This system, which has been used earlier and validated against visual observation, measures the total of body movements over a period of time, showing a large amplitude during intense body movements such as digging, rearing, grooming and exploration, and an almost zero amplitude during behavioural immobility. Animals were allowed to habituate to the recording cages for at least 2 h before the recordings started.

#### Experimental design

Animals were kept on the 12:12 light–dark cycle (light intensity: 60 lux) which constitutes the condition of entrainment. An overview of the design of the experiment is presented in Fig. 1. The light regime was changed after 10 days into constant dim light (5–6 lux), on which rats were maintained for the following 20 days. EEG
and to prevent synchronization.

conditions was done at irregular time points, just as the cleaning water and the addition of new rat chow in the constant dim light were always available ad libitum, the presentation of fresh identified off-line according to well-known criteria (van Luijtelaar with long lasting period of behavioural inactivity. The beginning of the activity bouts over days were used to estimate the beginning of the active period. Next the circadian day estimation, visual inspection of the activity rhythm over days shows marked which gives the accurate estimation of the period length. In addi-
tion representing the last 10 days of the 20 days spent in constant light condition was based on the outcomes of the Cosinor method, which yields also a measure of the robustness of the rhythm

The occurrence of SWD in the entrained phase showed also a clear, circadian rhythm. The minimum occurred during the first 2 h of the light phase. Then the number gradually increased to reach a maximum at the end of that phase. At the beginning of the dark phase a small drop occurred followed by an increase and then again a continuous decrease (Fig. 2). There were no differences in the number of SWD between the light and the dark phase of the 24-h rhythm, as well as between the active and the passive phase of the SWD rhythm recorded in the constant dim light condition. However, the number of SWD was higher in the active in comparison with the dark phase of the entrained condition (p < 0.05). The period of the SWD rhythm in constant dim light condition was shorter than 24 h (22.93 ± 0.53), although the difference just failed to reach significance. However, each animal showed a significant (it means that a significant amount of variation across time points was

### Figure 1

Experimental design.

motor activity recordings

EEG recordings

<table>
<thead>
<tr>
<th>0</th>
<th>10</th>
<th>23</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>entred condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>constant dim light condition</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sleep—wake states were identified off-line by visual inspection of the EEG and PIR recordings from the last day of the entrained and the last day of constant dim light condition, commonly used criteria were used (van Twyver, 1969; van Luijtelaar and Coenen, 1993). The second and penultimate hour of the dark and the light phase of 12:12 light—dark cycle and corresponding hours of the active and the passive phase of the constant dim light condition were analysed. Four sleep—wake states were scored: active wakefulness, passive wakefulness, slow-wave sleep and REM sleep. To determine the preference of the occurrence of SWD for a particular state of vigilance, every 5 s preceding and following each SWD were analysed. Results were subsequently corrected with regards to the distribution of the sleep—wake states over the analysed hours, as proposed by Drinkenburg et al. (1991).

### Statistical analysis

Statistical analysis were perform in Statistica (StatSoft, Inc.). Multivariate analysis of variance was first used to estimate differences in parameters between light regimes, only in case of significant condition effects, Student’s t-tests for dependent samples were used as post hoc tests. Differences were considered as significant at p < 0.05.

### Results

In the entrained condition (24 h, 12:12 light—dark), a rhythm of motor activity was found with a period length of 24.03 ± 0.04 h. Animals were more active during the dark phase than during the light phase of the 12:12 light—dark cycle (p < 0.001).

Multivariate analysis of variance showed significant differences between experimental condition for both motor activity and SWD rhythm (F = 84.84, df = 4.3, p < 0.01 and F = 10.91, df = 4.3, p < 0.05 respectively). Subsequent post hoc tests showed that the period of motor activity rhythm increased to 24.63 ± 0.08 h (p < 0.01), while the mean, amplitude and robustness of the rhythm decreased (all ps < 0.05) in the constant dim light. The number of activity counts was higher for the active than for the passive phase of the constant dim light condition (p < 0.001). A comparison between the entrained and the constant dim light condition revealed that animals were less active in the active phase and more active in the passive phase of the constant dim light condition (both ps < 0.05). Data are presented in Table 1.

In order to compare sleep, motor activity, SWD in the active and passive phases of the entrained and constant dim light periods, the beginning and the end of the active period in the entrained and constant dim light conditions was established. The onset of darkness was taken as the beginning of the active phase for the entrained condition. The onset of the activity rhythm in the constant dim light condition was based on the outcomes of the Cosinor method which gives the accurate estimation of the period length. In addition, visual inspection of the activity rhythm over days shows marked and long lasting activity bouts after a period of behavioural inactivity. The beginning of the activity bouts over days were used to estimate the beginning of the active period. Next the circadian day was splitted in two equal parts (the active and passive phase). It was noticed that the beginning of the passive period always coincided with long lasting period of behavioural inactivity.

EEGs were recorded during 4 days in the entrained condition and during 7 days of the constant dim light condition. SWD were identified off-line according to well-known criteria (van Luijtelaar and Coenen, 1986). The Cosinor analysis was applied on 4 and 7 day segments of the data collected in the entrained and constant dim light conditions respectively (data resolution: 6 min, number of SWD per 6 min block), to estimate the circadian period, mean, amplitude and robustness of the SWD rhythm.

### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Motor Activity Period</th>
<th>Mean</th>
<th>Amplitude</th>
<th>Robustness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entrained</td>
<td>24.63 ± 0.08 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dim Light</td>
<td>22.93 ± 0.53 h</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Preliminary PIR recordings were visually inspected to define an activity threshold value. The number of episodes above the threshold (= active behaviour) was counted automatically from the PIR recordings. Recordings were divided into 6 min bins. Each bin with a predominance (>50%) above the threshold was scored as 1. The value 0 was given to bins with a predominance of passive behaviour. Activity data were divided into two 10 day segments: the first of which represents 10 days spent in the entrained condition, the second representing the last 10 days of the 20 days spent in constant dim light conditions. The period, mean and amplitude of the activity rhythms of both segments were analysed by means of the Cosinor method, which yields also a measure of the robustness of the rhythm (the percentage of variance accounted for by the rhythm).

### Data analysis

Preliminary PIR recordings were visually inspected to define an activity threshold value. The number of episodes above the threshold (= active behaviour) was counted automatically from the PIR recordings. Recordings were divided into 6 min bins. Each bin with a predominance (>50%) above the threshold was scored as 1. The value 0 was given to bins with a predominance of passive behaviour. Activity data were divided into two 10 day segments: the first of which represents 10 days spent in the entrained condition, the second representing the last 10 days of the 20 days spent in constant dim light conditions. The period, mean and amplitude of the activity rhythms of both segments were analysed by means of the Cosinor method, which yields also a measure of the robustness of the rhythm (the percentage of variance accounted for by the rhythm).

In order to compare sleep, motor activity, SWD in the active and passive phases of the entrained and constant dim light periods, the beginning and the end of the active period in the entrained and constant dim light conditions was established. The onset of darkness was taken as the beginning of the active phase for the entrained condition. The onset of the activity rhythm in the constant dim light condition was based on the outcomes of the Cosinor method which gives the accurate estimation of the period length. In addition, visual inspection of the activity rhythm over days shows marked and long lasting activity bouts after a period of behavioural inactivity. The beginning of the activity bouts over days were used to estimate the beginning of the active period. Next the circadian day was splitted in two equal parts (the active and passive phase). It was noticed that the beginning of the passive period always coincided with long lasting period of behavioural inactivity.

EEGs were recorded during 4 days in the entrained condition and during 7 days of the constant dim light condition. SWD were identified off-line according to well-known criteria (van Luijtelaar and Coenen, 1986). The Cosinor analysis was applied on 4 and 7 day segments of the data collected in the entrained and constant dim light conditions respectively (data resolution: 6 min, number of SWD per 6 min block), to estimate the circadian period, mean, amplitude and robustness of the SWD rhythm.
Endogenous control of SWD rhythm

Table 1  Mean and SEM of the various parameters of the motor activity rhythm in the entrained and constant dim light conditions.

<table>
<thead>
<tr>
<th></th>
<th>Entrained</th>
<th>Constant dim light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (h)</td>
<td>24.03 ± 0.04</td>
<td>24.63 ± 0.08*</td>
</tr>
<tr>
<td>Mean(^a)</td>
<td>0.20 ± 0.03</td>
<td>0.16 ± 0.06*</td>
</tr>
<tr>
<td>Amplitude</td>
<td>0.18 ± 0.01</td>
<td>0.11 ± 0.01*</td>
</tr>
<tr>
<td>Robustness(^b) (%)</td>
<td>8.58 ± 1.59</td>
<td>4.28 ± 0.64*</td>
</tr>
</tbody>
</table>

\(^a\) An estimate of central tendency of the distribution of values of an oscillating variable.
\(^b\) The percentage of variance accounted for by the rhythm.
* Entrained vs constant dim light \(p < 0.05\).

Table 2  Mean and SEM of the various parameters of the SWD rhythm in the entrained and constant dim light conditions.

<table>
<thead>
<tr>
<th></th>
<th>Entrained</th>
<th>Constant dim light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (h)</td>
<td>24.00 ± 0.04</td>
<td>22.93 ± 0.53</td>
</tr>
<tr>
<td>Mean(^a)</td>
<td>1.41 ± 0.18</td>
<td>1.82 ± 0.15*</td>
</tr>
<tr>
<td>Amplitude</td>
<td>5.45 ± 0.48</td>
<td>8.32 ± 0.68*</td>
</tr>
<tr>
<td>Robustness(^b) (%)</td>
<td>4.44 ± 0.70</td>
<td>1.57 ± 0.29*</td>
</tr>
<tr>
<td>SWD number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark/active</td>
<td>631.43 ± 81.08</td>
<td>919.28 ± 65.89*</td>
</tr>
<tr>
<td>Light/passive</td>
<td>738.43 ± 77.38</td>
<td>865.71 ± 90.38</td>
</tr>
</tbody>
</table>

\(^a\) An estimate of central tendency of the distribution of values of an oscillating variable.
\(^b\) The percentage of variance accounted for by the rhythm.
* Entrained vs constant dim light \(p < 0.05\).

explained by the fitted cosinus), circadian rhythm in the occurrence of SWD under the constant dim light condition. Significant changes were also found for the mean and amplitude of the rhythm, which were larger in the constant dim light condition \((p < 0.05; p < 0.001\) respectively), as well as for the robustness, which was smaller than in the entrained condition \((p < 0.05\). Results are presented in Table 2.

The comparison of parameters of the activity and SWD rhythm in the constant dim light condition revealed significant differences. The period length of the SWD rhythm was shorter and the rhythm less robust \((p's < 0.05\), while the amplitude and the mean of the SWD rhythm was higher in comparison to the activity rhythm (both \(p's < 0.001\).

The distribution of particular states of vigilance in the entrained condition is presented in Table 3. Active wakefulness, dominating at the beginning of the dark phase, decreased gradually till the beginning of the light phase, in which it reached its lowest level. At the end of the light phase the amount of active wakefulness slightly increased. The same pattern was also observed for passive wakefulness, while the course of the slow-wave sleep was opposite. The lowest amount of slow-wave sleep was observed at the beginning of the dark phase, subsequently followed by a gradual increase till the beginning of the light phase when it reached its maximum. Contrary to active and passive wakefulness, the amount of slow-wave sleep decreased at the end of the light phase. The percentage of REM sleep gradually increased during the investigated hours.

Active wakefulness in the constant dim light condition was found to be the only state of vigilance, which preserved its pattern observed in the entrained condition. The course of the remaining sleep—wake states was altered. The lowest amount of passive wakefulness was found at the end of the active phase, followed by an increase at the beginning of the passive phase and a subsequent decrease at the end. A minimal amount of slow-wave sleep was recorded, the same as in the entrained condition, at the beginning of the active phase. The maximum was shifted from the beginning of the passive phase to the end of the active phase, after which a gradual decrease was observed. A slight alteration was also found for REM sleep. The same pattern for the three investigated periods (the entire active phase and the beginning of the passive phase) was preserved, with

Figure 2  Double plotted graph of 4 days of SWD (mean number and SEM of SWD per hour) activity (one X-axis represents two days (48 h), the activity of the second day is plotted again as the first day in the next X-axis). The dark phase of the 12:12 light—dark cycle is marked in grey.

Figure 3  Double plotted graph of 4 days of SWD (mean number and SEM of SWD per hour) activity (one X-axis represents two days (48 h), the activity of the second day is plotted again as the first day in the next X-axis). The dark phase of the 12:12 light—dark cycle is marked in grey.

Figure 4  Double plotted graph of 4 days of SWD (mean number and SEM of SWD per hour) activity (one X-axis represents two days (48 h), the activity of the second day is plotted again as the first day in the next X-axis). The dark phase of the 12:12 light—dark cycle is marked in grey.
Table 3  Mean and SEM of the percentage of particular states of vigilance during investigated hours in the entrained and constant dim light conditions.

<table>
<thead>
<tr>
<th></th>
<th>Dark phase</th>
<th>Light phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning</td>
<td>End</td>
</tr>
<tr>
<td>Entrained condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active wakefulness</td>
<td>60.22 ± 3.61</td>
<td>38.21 ± 9.22</td>
</tr>
<tr>
<td>Passive wakefulness</td>
<td>18.26 ± 1.96</td>
<td>13.08 ± 3.38</td>
</tr>
<tr>
<td>Slow-wave sleep</td>
<td>19.10 ± 3.64</td>
<td>40.70 ± 8.54</td>
</tr>
<tr>
<td>REM sleep</td>
<td>2.42 ± 1.22</td>
<td>8.01 ± 3.21</td>
</tr>
<tr>
<td>Constant dim light condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active wakefulness</td>
<td>54.16 ± 7.41</td>
<td>26.54 ± 8.88</td>
</tr>
<tr>
<td>Passive wakefulness</td>
<td>12.41 ± 1.14</td>
<td>6.77 ± 1.63</td>
</tr>
<tr>
<td>Slow-wave sleep</td>
<td>30.26 ± 5.92</td>
<td>59.65 ± 7.23</td>
</tr>
<tr>
<td>REM sleep</td>
<td>3.17 ± 2.01</td>
<td>7.04 ± 2.10</td>
</tr>
</tbody>
</table>

The exception of the end of the passive phase in which a decrease was observed. The distribution of the particular states of vigilance recorded in the constant dim light condition is presented in Table 3.

A comparison made between the entrained and the constant dim light condition revealed that in the active phase of the constant condition the percentage of passive wakefulness was lower ($p < 0.05$). In the passive phase of the constant dim light condition the percentage of active wakefulness was higher ($p < 0.05$), whereas the percentage of slow-wave sleep was lower ($p < 0.05$).

Passive wakefulness and slow-wave sleep have been found to dominate among states of vigilance preceding the occurrence of SWD in the entrained condition. 39% and 35% of SWD recorded were preceded by passive wakefulness and by slow-wave sleep respectively, active wakefulness (15%) and REM sleep (11%) were the least probable states. This tendency was preserved in the constant dim light condition. 47% of SWD were preceded by passive wakefulness, 32% by slow-wave sleep, 17% by active wakefulness and 4% by REM sleep. No significant differences between the entrained and the constant dim light condition were found. Data are presented in Fig. 3.

Passive wakefulness in the entrained condition followed the occurrence of SWD most frequently (71%), active wakefulness, with a probability of 17%, was the second state.

Figure 3  States of vigilance preceding (A and C) and following (B and D) the occurrence of SWD in the entrained (A and B) and constant (C and D) conditions. AW = active wakefulness, PW = passive wakefulness, SWS = slow-wave sleep, REM = REM sleep. SWS after SWD occurred more often in the constant dim light condition, *$p < 0.05$. 
Slow-wave sleep and REM sleep were recorded after SWD in 9% and 3% of cases respectively. The same relationship was found in the constant dim light condition for two sleep—wake states: passive wakefulness and REM sleep. Passive wakefulness was the most and REM sleep the least probable to occur after SWD (62% and 9% respectively). Opposite to the entrained condition, slow-wave sleep was found to be the second most frequent state following the occurrence of SWD (18%), active wakefulness was the third (11%). Data are presented in Fig. 3.

Statistical analysis revealed that the probability that SWD was followed by slow-wave sleep is significantly higher in the constant dim light condition than in the entrained condition (p < 0.05).

Discussion

In the present study the rhythm of absence seizures, the rhythm of general motor activity and the relationship between absence seizures and states of vigilance in WAG/Rij rats were investigated under two different experimental conditions: the condition of entrainment to the 12:12 light—dark cycle, and the condition of constant dim light. Here it is shown for the first time that there is an endogenous rhythm of absence seizures, given that the circadian rhythmicity in the occurrence of SWD is still present in the absence of the most important Zeitgeber. Moreover, under constant dim light condition significant changes in various parameters of the rhythm of SWD and in general motor activity are observed, while additionally internal desynchronization between these rhythms is observed. Contrary to these changes, the well established relationship between the occurrence of SWD and states of vigilance is not altered by the constant environment. Regardless of environmental conditions, SWD are most frequently occurring during passive wakefulness and slow-wave sleep.

The present results confirm previous observations of circadian rhythmicity in rats' motor activity (Buttner and Wollnik, 1984; Ray et al., 2004; Refinetti, 2006). Animals are more active during the dark than during the light phase in the entrained condition. The constant dim light alters parameters of the motor activity rhythm. The period of the rhythm becomes longer than 24 h, in agreement with "Aschoff's rule" stating that, in nocturnal animals maintained on constant light, an increase in light intensity corresponds with an increase in the period length of the rhythm. It can be concluded that a slight, but significant, increase in period length observed in the present experiment is caused by the presence of constant low intensity light on which animals are maintained (Aschoff, 1960; Daan and Pittendrigh, 1976; Fukuhara et al., 2005). The remaining parameters such as the mean, amplitude and robustness of the rhythm are found to be decreased in comparison with the condition of entrainment. An inhibitory effect of light on the activity of nocturnal animals is well established (Redlin, 2001). A long-lasting, continuous exposure to dim light has been reported to reduce the amplitude and the mean of activity rhythm with a possible disruption of the circadian pattern (Borbély and Neuhaus, 1978; Albers et al., 1981; Eastman and Rechtschaffen, 1983; Ikeda and Inoue, 1998).

A circadian rhythmicity in the occurrence of SWD in WAG/Rij rats is found in the entrained condition. The observed pattern shares common characteristics with previous reports (van Luijtelaar and Coenen, 1988). The circadian distribution is a direct consequence of the changes in sleep—wake states over the course of the 24-h day (Drinkenburg et al., 1991). Light slow-wave sleep, passive wakefulness and transitions between different states of vigilance have been shown to promote the occurrence of SWD, while deep slow-wave sleep, REM sleep and active wakefulness are states exerting opposite effects (Lannes et al., 1988; Coenen et al., 1991; Drinkenburg et al., 1991). In both studies the nadir of the rhythm was found to be located during the first hours of the light phase, which correlates with a high amount of deep slow-wave sleep at that time of the light—dark phase (Coenen et al., 1991; Drinkenburg et al., 1991). A slight difference was noted for the maximum of the rhythm: it occurred at the end of the light phase, while van Luijtelaar and Coenen (1986) pointed out that this maximum emerged at the early hours of the dark phase. Despite the high number of SWD in the dark phase, a decline was observed just at the beginning of it. For nocturnal animals kept in laboratory conditions, turning off the light is a strong signal to become behaviourally active. Therefore, the characteristic peak of activity at the beginning of the dark period could be responsible for such a decline, as active wakefulness suppresses the occurrence of SWD (Drinkenburg et al., 1991; Coenen et al., 1991).

The changes in the SWD rhythm that took place in constant dim light condition show an opposite direction in comparison with changes in the rhythm of motor activity. The period length of the absence epilepsy rhythm becomes shorter than 24 h. Although this change is not significant according to parametric tests for the group as unity, each animal showed a clear circadian rhythm with a new, shorter period length in the range of 20.6—23.9 h. A non-parametric sign test confirmed that the period length was indeed shorter in constant dim light. A circadian rhythm of limbic seizures was first described by Quigg et al. (2000) in a model for temporal lobe epilepsy: the seizures occurred in a phasic nonrandom pattern both under entrainment and under constant darkness with a period length of about 24 h. Similar to the present results, the number of seizures, as well as the amplitude of the rhythm increased in an environment lacking external time cues.

Contrary to a decrease in the amplitude and in the mean of the motor activity rhythm, the corresponding parameters of the rhythm of SWD increased. Motor activity and waking are suppressed in the condition of continuous light (Borbély and Neuhaus, 1978). This suppression, can be a putative cause for the increase in the number of SWD, considering the intimate negative relationship between vigilance and arousal, and the occurrence of SWD. Moreover, it has been shown that the condition of constant light disrupts the sleep—wake pattern. A significant increase of slow-wave and REM sleep is found under constant light, together with an elevated number of REM sleep episodes (Borbély and Neuhaus, 1978). Similar results were obtained by Ikeda et al. (2000), additionally pointing towards a redistribution of the amount of particular sleep stages in the active and the passive phase. In the present study, dim light alters the course, as well as the amount of sleep—wake states.
The divergence of the period length under constant dim light between motor activity, with a significant increase, and the SWD rhythm, with a tendency to decrease, suggests distinct oscillators, responsible for their regulation. Others have found dissociation between circadian rhythms in continuous dim light in monkeys and rats (Erket, 2000; Aguzzi et al., 2006). Studies devoted to the process of desynchronization of oscillators are considered as important evidence in multiple oscillatory theory of mammalian circadian timing system. These oscillators may reside in or outside the SCN (Pittendrigh and Daan, 1976; Shinohara et al., 1995; Jagota et al., 2000).

A common finding for both the motor activity and the SWD rhythm is a gradually decreasing robustness after the transfer to the constant dim light condition. Both rhythms show the same tendency, however, the motor activity rhythm was found to be significantly more robust throughout the whole experiment than the SWD rhythm. This suggests that the motor activity rhythm is governed by a stronger oscillator. Differences in robustness of the rhythms of wake and body temperature under long-term continuous illumination were earlier reported: the wake rhythm, thought to be driven by a weaker oscillator, lost its organization earlier than the body temperature rhythm (Eastman and Rechtschaffen, 1983). Although the 22.9 h rhythm of SWD remained identifiable, it was also noticed that it became less organized. The motor rhythm keeps its organization better. Both rhythms showed a diminished robustness, reflecting a decrease in the strength of the circadian timing system. Therefore it seems that under entrained conditions, the structure of the SWD rhythm is influenced by changes in sleep—wake states and in the amount and timing of activity over the 24-h day (Coenen et al., 1991; Drinkenburg et al., 1991). A disrupted organization of general motor activity, expressed by a decreased robustness of its rhythm, together with a distinct oscillator acting independently of the oscillator controlling the timing of SWD, might be responsible for the gradual change of the structure of SWD rhythm under constant dim light conditions.

In the present study, the majority of SWD was preceded by passive wakefulness and slow-wave sleep, while active wakefulness and REM sleep were rarely recorded just before SWD, consistent with previous findings from both animal and human studies (Lannes et al., 1988; Drinkenburg et al., 1991; Halasz et al., 2002). The relationship between SWD and states of vigilance are the same in both the entrained and constant dim light condition, strongly suggesting that the dependency of generation of SWD on particular state of vigilance is not influenced by circadian factors.

SWD are often followed by active and passive wakefulness, this awakening effect of SWD observed in the present experiment supports earlier findings (Drinkenburg et al., 1991). Here it is found that the probability that SWD are followed by slow-wave sleep, is higher in constant dim light than in entrained condition, while the sleep—wake state preceding the onset of SWD is not changed. This implies that the awakening properties of SWD are diminished in constant dim light condition. The sleep—wake cycle of these epileptic rats has been found to be disrupted: besides shorter and many aborted REM sleep periods (Suntsova et al., 2009; Gandolfo et al., 1991), the sleep cycle is shortened. However, the sleep cycle disturbances are significant only at a specific time of day, pointing towards a circadian factor in sleep disturbances (van Luijtelaar and Bikbaev, 2007). It seems that the tendency of SWD to exert an awakening effect is reduced by uncoupling the SWD and motor rhythms and by a decrease in the robustness of these rhythms.

In conclusion, this study demonstrates that the timing of SWD under entrained conditions is due to photic synchronizing stimuli and shaped by the motor rhythm and the momentary state of vigilance. It also confirmed a true circadian nature of the rhythm of absence seizures. Considering an opposite tendency in the characteristics of rhythms under constant dim light conditions, it can be concluded that the SWD rhythm might be driven by a circadian oscillator distinct from that of a general motor activity rhythm. The relationship between states of vigilance and the mechanisms of the generation of SWD is preserved in the constant dim light condition suggesting, that this mechanism is independent from circadian modulation. However, the awakening effect of SWD became weaker. The uncoupling of SWD and motor rhythm during constant dim light conditions allows that the SWD rhythm looses some of its robustness.

Acknowledgements

The study is supported by a fellowship from Radboud University Nijmegen. The authors would like to thank Gerard van Oijen, Pascal de Water for technical and Saskia Hermeling and Hans Krijnen for biotechnical assistance.

References


Langdon-Down, M., Brain, W., 1929. Time of day in relation to convulsions in epilepsy. Lancet, 1029–1032.


