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Spike–Wave Discharges and Sleep–Wake States during Circadian Desynchronization: No Effects of Agomelatine upon Re-Entrainment

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Abstract—Rapid changes in the light–dark cycle cause circadian desynchronization between rhythms of spike–wave discharges (SWDs) and motor activity in genetic epileptic rats, and this is accompanied by an increase in epileptic activity. Given the close relationship between absence seizures and sleep–wake states, the present study assessed firstly a putative relationship between vigilance rhythms and SWDs during re-synchronization, and secondly sleep–wake patterns responsible for increased epileptic activity. Lastly, in a view of existing evidence that melatonin and its agonists accelerate re-synchronization, the effects of different doses of agomelatine upon the speed of re-synchronization of different sleep–wake states and SWDs were investigated. Simultaneous electroencephalographic and electromyographic recordings were made in symptomatic WAG/Rij rats, before, during and 10 days following an 8 h light phase delay. Agomelatine was orally administered acutely and sub-chronically, during 10 post-shift days. The magnitude of the advance after the shift and the speed of re-synchronization were specific for various rhythms. Most prominent change was the increase in REM sleep duration during the dark phase. A post-shift increase in passive wakefulness and a reduction in deep slow-wave sleep coincided with an aggravation of SWDs during the light phase. Agomelatine showed neither an effect on sleep–wake parameters and SWDs, nor affected re-synchronization. The same speed of re-synchronization of SWDs and light slow-wave sleep suggests that both are controlled by a common circadian mechanism. The redistribution of SWDs and their increase in the light phase after the shift may be of importance for patients with absence epilepsy planning long trans-meridian flight across time zones. © 2019 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: absence epilepsy, WAG/Rij rats, sleep–wake cycle, jet lag, phase delay, agomelatine.

INTRODUCTION

Survival of the organism, successful adaptation to a particular ecological niche, and well-being of the organism are all ensured by a proper synchronization of the circadian timing system to the environmental cycles (Dodd et al., 2005). Rapid shifts in the timing of the light–dark cycle, one of the most powerful circadian synchronizers or so-called “Zeitgebers”, lead to circadian desynchronization described in jet travelers crossing more than two time zones, in shift workers and in animal models (Arendt and Marks, 1982; Knutsson, 2003;

Nagano et al., 2003). Re-synchronization after a shift needs a considerable amount of time, different for various functions and rhythms (Yamazaki et al., 2000; Lemmer et al., 2002). Exogenous melatonin accelerates re-synchronization and therefore ameliorates symptoms of jet lag syndrome both in humans and animals (Illnerová et al., 1989; Golombek and Cardinali, 1993; Sharkey and Eastman, 2002).

The consequences of internal desynchronization in WAG/Rij rats, a well-known, validated, genetic animal model of childhood absence epilepsy (Depaulis and van Luijtelaar, 2006), have been previously described (Smyk et al., 2011, 2012). The occurrence of absence seizures in this strain of rats, marked by the presence of short-lasting, bilateral and synchronous 7–11 Hz spike–wave discharges (SWDs) in the electroencephalogram (EEG), is organized in a 24 h rhythm (van Luijtelaar and Coenen, 1988). In a constant dim light condition and after an 8 h light phase delay, the

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rhythm of SWDs was found to desynchronize from the rhythm of motor activity (Smyk et al., 2011, 2012). Opposite to the gradual and stable re-synchronization of the motor activity to the shifted photoperiod that was completed after 6 days, re-synchronization of the rhythm of SWDs took longer and developed in an irregular way. Moreover, the lack of internal synchrony of rhythms was associated with an enhancement of epileptic activity (Smyk et al., 2011, 2012). Considering a close relationship between the occurrence of absence seizures and sleep–wake states (Drinkenburg et al., 1991), we aimed to determine to which of the sleep–wake state rhythms, if any, SWDs are coupled during the re-synchronization process.

A second goal of the present study was to investigate the putative effects of sub-chronic administration of several doses of agomelatine on the course of re-synchronization of the rhythms of SWDs and sleep–wake states to an 8 h phase delay. Agomelatine, a newly introduced antidepressant with chronobiotic properties, is a potent agonist of melatonin receptors subtypes 1 and 2 (MT₁, MT₂) and a weak antagonist of the serotonin receptor subtype 2 (5-HT_{2C}) (de Bodinat et al., 2010). The antidepressant action of agomelatine has been demonstrated in animal studies and in clinical trials (Papp et al., 2003; Goodwin et al., 2009). Chronobiotic properties of the drug have been reported in rats kept in constant darkness (Martinet et al., 1996), in an 8 h phase advance paradigm resembling jet-lag (Redman et al., 1995), and in aged rodents (Koster-van Hoffen et al., 1993).

Melatonin and agomelatine are known to affect epileptic activity in diverse seizure and epilepsy models such as pentylenetetrazole, pilocarpine, kainite, quinolinate, glutamate and NMDA models in mice (Lapin et al., 1998; Yildirim and Marangoz, 2006; Aguiar et al., 2012; Dastgheib and Moezi, 2014), as well as penicillin-induced and absence seizures in rats (Aygun et al., 2015; Ethemoglu et al., 2019). Although ineffective against epileptogenesis induced in the kainic acid-induced status epilepticus model, agomelatine given chronically prevents neuronal damage and comorbid depression in rats (Thekalarova et al., 2017 and 2018). Melatonin and agomelatine may also alter sleep–wake architecture in the rat (Descamps et al., 2009). Considering the potential hypnotic and antiepileptic effect of agomelatine, our sub-chronic experiment was preceded by an acute study, which enabled us to disentangle the putative effects of agomelatine on sleep and epilepsy from the effects of the light phase delay on re-synchronization. In the acute experiment the effects of a single administration of several doses of agomelatine on the number and mean duration of SWDs, on sleep–wake states and circadian parameters of their rhythms were investigated.

EXPERIMENTAL PROCEDURES

Animals

Thirty-two male, eight-months old WAG/Rij rats (Harlan, the Netherlands), average weight 315 g ± 12 g at time of surgery, were used. All protocols have been carried out in accordance with guidelines of the Association for Assessment and

Accreditation of Laboratory Animal Care International (AAALAC), and of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Janssen Pharmaceutica Ethical Committee. Every effort was made to minimize animal use and disturbances in animal well-being.

Rats were singly-housed in individually ventilated Plexiglas cages (25 × 22 × 18 cm) in a sound-attenuated room and controlled environmental conditions throughout the experiment as well as during the recovery period after surgery. Environmental parameters were as follows: temperature: 22 ± 2 °C; humidity: 60%; 12:12 light–dark cycle, light intensity: ≈ 100 lx, (i.e. at the height of the recording box, while a recessed lighting consoles managed diffuse and uniform light levels below 60 lx within cages, a software-controlled dimmer handled a gradual transition between light and dark cycles). Standard laboratory chow (SAFE A04, Augy, France) and tap water were available ad libitum.

All experiments were performed in a large scale EEG laboratory setting under controlled conditions, in which animals were kept in their home cage and placed in individually ventilated recording boxes 24 h before the start of the first baseline recording and throughout the sub-chronic experiments in order to avoid any stress that may result from cage changes and displacement of home-cages from the holding room to recording room and vice-versa.

Rats were euthanized at the end of the study by a conventional rodent CO₂ euthanasia procedure.

Surgery

Surgery was performed using the protocol described earlier (Ahnaou et al., 2009). Rats, under isoflurane anesthesia, were equipped with electrodes for EEG and EMG recordings. A mixture of 30% O₂, 70% N₂O and 5% isoflurane was administered to animals as an initial induction for 2 min. Then, the animals were mounted in a stereotaxic apparatus and were given a continuous constant mixture of O₂, N₂O and 2% isoflurane. The analgesic Piritramide (Dipidolor; Janssen Pharmaceutica NV, Belgium) was administered before the incision over the total length of the head. The oval area of the scalp was removed, and the uncovered skull was cleared of the periosteum in order to place 7 stainless steel fixing screws (diameter 1 mm), inserted bilaterally in the left and right hemisphere along the antero-posterior axes in the following coordinates: AP +2 mm, L +/−2 mm; AP −2 mm, L +/−2 mm and AP −6.6 mm, L +/−2 mm from Bregma; and referenced to the same ground electrode place midline above of the cerebellum. The incisor bar was −5 mm under the center of the ear bar, according to the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 2013). In addition, stainless steel wire electrodes were placed in the muscles of the neck to record EMG activity. Electrodes (stainless steel wire, 7N51465T5TLT, 51/46 Teflon Bilaney, Germany) were connected to a pin (Future Electronics: 0672-2-15-15-30-27-10-0) with a small insert (track pins; Dataflex: TRP-1558-0000) and were fit into a 10-holes connector after which the whole assembly was fixed to the cranium with dental cement. Rats were allowed to recover for at least 10 days. Welfare of each

animal was monitored by an animal inventory system with regular observations of behavior and physical health.

EEG and EMG recordings

During the adaptation period and recordings, animals were carefully connected via a rotating swivel allowing free movement during recording procedures. Continuous EEG and EMG signals were acquired at 2 kHz sample rate with an input range of ± 500 mV through a Biosemi ActiveTwo system (Biosemi, Amsterdam, the Netherlands) referenced to the CMS-DRL ground (common mode reference for online data acquisition and impedance measures, which is a feedback loop driving the average potential across the montage close to the amplifier zero). The signals were amplified, analogue band-pass filtered between 1 and 100 Hz and digitized with 24-bit resolution. Steel wire electrodes were placed in the muscles of the neck to record EMG activity. These procedures and instrumentation have shown to be reliable for long-term, high-quality EEG and sleep–wake organization analysis (Ahnaou et al., 2015).

Pharmacological treatment

Saline, agomelatine (Sigma-Aldrich, Belgium) in doses of 2.5, 5 and 10 mg/kg dissolved in 20% cyclodextrin (Sigma-Aldrich, Belgium) and 20% cyclodextrin alone for vehicle control condition were always administered orally (in < 10 ml/kg volume) at 1 h before the onset of the dark period.

Experimental design

The acute study consisted of three experimental days. Each day consisted of 24 h of simultaneous and continuous EEG and EMG recordings. Recordings started at the last hour of the light phase during which administrations took place, next they were continued by 12 h during the dark phase and 11 h during the light phase (light–dark switch via dusk-dawn transition of 10 min). Animals were randomly assigned to 4 conditions: vehicle, 2.5, 5 and 10 mg/kg group of agomelatine ($n = 8$ rats each condition). On the first, baseline day, all rats received saline as a within-subject control for and adaptation to the administration procedure. On the second day, the treatment day, animals received agomelatine or vehicle (control condition). On the third, post-treatment day, oral administration of a compound was mimicked by picking up animals from their cages without usage of the gavage tubes and pharmaceutical agents.

Next, the sub-chronic study consisted of 11 experimental days and one period of extended darkness. Recording schedule was the same as in the acute study design. Again, rats were randomly assigned to 4 experimental groups ($n = 8$ rats each): vehicle, 2.5, 5 and 10 mg/kg of agomelatine. On the first baseline day, all animals received saline 1 h before the start of the dark phase. After that day, an 8 h phase shift in the light–dark cycle was introduced by extending the subsequent dark period from 12 to 20 h. Recordings were continued for 10 consecutive, post-shift days. Every day, 1 h before the dark phase, animals were given either agomelatine or vehicle (control group). Due to an unexpected

recording system failure, the data of the second post-shift day were lost and are treated as ‘missing data’ in the analysis. Experimental design is presented in Fig. 1.

The acute and sub-chronic study were separated by a two-week washout period.

Data analysis

Five sleep–wake states were automatically identified for each 2 s epochs: active wakefulness, passive wakefulness, light and deep slow-wave sleep and rapid-eye-movement sleep (REM), based on EEG, EMG and body movements (PIR detection) following validated criteria (Ahnaou and Drinkenburg, 2011).

The number and duration of SWDs was scored automatically based on the EEG signal from a frontal electrode against reference according to validated criteria, next SWDs were visually scored based on criteria published elsewhere (Ovchinnikov et al., 2010).

Data from 24 h days of both acute and sub-chronic experiment: duration of the five sleep–wake states (expressed in min.), number and mean duration of SWDs (duration expressed in sec.) were grouped into 1 h bins. Each hour of the baseline day was compared with the corresponding hours of the treatment and post-treatment days in the acute, and 10 post-shift days in the sub-chronic study, respectively.

The acrophases of active wakefulness, both slow-wave sleep stages and SWDs rhythms, were based on 6 min bin data, as previously described (Smyk et al., 2011, 2012). Cosine wave of a fixed 24 h period length was fitted to each of the 3 days of the acute study, baseline and all post-shift days of the sub-chronic study (program Cosinor available at <http://www.circadian.org/software.html>). Considering that the shape of the passive wakefulness and REM sleep rhythms during re-synchronization diverged significantly from cosine wave, their acrophases were assessed as follows: a position of a peak for each day was determined visually based on 0.5 h bin data. Additionally, the timing of minima of those rhythms was assessed as well. Also time series consisting of all experimental days for all sleep–wake states and SWDs (bin size: 6 min) were constructed and correlated with each other to reveal the strength of the mutual relationships.

Statistical analysis

Statistical analyses were done in Statistica (StatSoft, Inc., Tulsa, OK, USA). Repeated measures analysis of variance (ANOVA) were used to estimate dose, hour and day effect of agomelatine treatment, on total duration of the five sleep–wake states, mean duration and number of SWDs in the acute study. The same variables were also determined in the sub-chronic experiment in which, dose, phase (light and dark) and day effects were investigated. Repeated measures ANOVA were also used to estimate the dose and day effect of agomelatine treatment on circadian parameters of the rhythms of sleep–wake states and SWDs in both studies. Bonferroni's test was used as post-hoc test. The significant F values and their df are reported, followed by P values of the post-hoc tests. Alternatively, Friedman ANOVA followed by Dunn's multiple comparison test was used when the criterion

Fig. 1. Experimental design. EEG, EMG and activity were simultaneously recorded in symptomatic WAG/Rij rats injected with the melatonergic antidepressant agomelatine (A) in an acute (B) and sub-chronic experiment (C).

of the normality of the data was not fulfilled. Pearson's correlation coefficients (r) were calculated to compare strength of relationships between various rhythms throughout the sub-chronic experiment. Differences were considered to be significant at $P < .05$.

RESULTS

Acute study

No dose effect of agomelatine or interactions of agomelatine with days or hours were found on any variable investigated, neither in the duration of sleep–wake states, number and mean duration of SWDs or in any circadian parameters. Only hour, day, and hour $\times$ day effects were found. However, the magnitude of changes was small (in a range of 0.33–0.70 min per 24 h in case of the duration of sleep–wake states and 6.90–21 degrees per 360 degrees in case of the acrophase, on the treatment versus baseline day). An 'hour $\times$ day' interaction effect was found for the number of SWDs ($F_{\text{HOUR} \times \text{DAY}} = 8.18$, all $df = 46$, 1288); post-hoc tests (all $P < .05$) showed that the number of SWDs was decreased for four consecutive hours (4th, 6th–8th) on the treatment day compared to baseline and post-treatment day (Fig. 2).

Sub-chronic study

Dose effect or interactions of dose of **agomelatine** were not found during the process of re-synchronization on any variable investigated.

As a consequence of the phase shift, the total duration of **active wakefulness** was reduced on 1st, 3rd and 4th day after the shift ($F_{\text{DAY}} = 10.28$, $df = 9$, 504, all $P < .05$). Active wakefulness was decreased in the dark phase from 1st to 7th post-shift day, and increased in the light period ($F_{\text{DAY} \times \text{PHASE}} = 243.24$, $df = 9$, 504, all $P < .05$). Data are presented in Fig. 3.

During most post-shift days the total duration of **passive wakefulness** was elevated across days ($F_{\text{DAY}} = 6.63$, $df = 9504$, all $P < .05$). A robust increase was observed in the light phase from 1st to 4th, 5th and 7th post-shift day, while a nocturnal level was decreased on the 1st and 3rd post-shift day and increased during the last 3 days of the experiment ($F_{\text{DAY} \times \text{PHASE}} = 54.51$ $df = 9504$, all $P < .05$) (Fig. 3).

Changes of the total time spent in **light slow-wave sleep** in the light and the dark phase across days were significant ($F_{\text{DAY}} = 3.18$, $df = 9$, 504, $P < .05$), although they seemed less pronounced in comparison to active and passive wakefulness. Both diurnal and nocturnal level of light slow-wave sleep was increased and reduced, respectively only for

Fig. 2. Duration (minutes) of sleep-wake states (mean $\pm$ SEM, $n = 32$) and occurrence (number) of SWDs (mean $\pm$ SEM, $n = 32$) across 24 h during saline (A), treatment with various doses of agomelatine (B), and post-treatment day (C). Comparison between experimental days of the acute study (D). Data resolution: 1 h. The dark phase of the 12:12 light–dark cycle is marked by gray rectangles. * $P < .05$, baseline vs treatment day, # $P < .05$, baseline vs post-treatment day, ANOVA for repeated measures, Bonferroni post-hoc test.

2 days after the phase shift ($F_{\text{DAY} \times \text{PHASE}} = 12.66$, $df = 9$, 504, all $P < .05$) (Fig. 3).

Total duration of **deep slow-wave sleep** was significantly elevated in the dark phase during the first 5 days after the phase-shift, while its duration in the light phase was decreased for 4 days ($F_{\text{DAY} \times \text{PHASE}} = 472.70$, $df = 9504$, all

$P < .05$). On the first post-shift day, typical phase-related differences were noticed: the prolonged duration of deep slow-wave sleep during the dark was compensated by a firm reduction during the light phase ($P < .05$) (Fig. 3).

The duration of **REM sleep** was increased starting from the 1st to 6th post-shift day ($F_{\text{DAY}} = 12.71$, $df = 9504$, all

$P < .05$). This increase is completely due to the elevated level of REM sleep in the dark phase ($F_{\text{DAY} \times \text{PHASE}} = 28.87$, $df = 9504$, $P < .05$) (Fig. 3).

Rats had more **SWDs** in the light phase of the first 3 post-shift days in comparison to baseline, while during the dark phase of the same post-shift days SWDs were less numerous ($F_{\text{DAY} \times \text{PHASE}} = 21.04$, $df = 9, 36$, all $P < .05$). The mean duration of SWDs was increased on 3rd and 4th post-shift days ($F_{\text{DAY}} = 3.87$, $df = 9, 36$, all $P < .05$), the increase in the light phase was present during the first 3 post-shift days ($F_{\text{DAY} \times \text{PHASE}} = 3.28$, $df = 9, 36$, all $P < .05$) as the main contributor to this effect (Fig. 3). There were no dose effects or interactions with dose of agomelatine on number and mean duration of SWDs.

Circadian rhythmicity was detected in all sleep–wake states and the occurrence of SWDs on the baseline day.

In general, dose effect of agomelatine and its interactions were not found in acrophases of any of the rhythms investigated. To avoid that the outcomes of circadian analyses were overpowered by the large number of animals ($n = 32$), only the data from the control group ($n = 8$) were used to illustrate the process of re-entrainment of various rhythms to the phase shift.

The **position of acrophase** changed over days in all states and SWDs: active wakefulness: $F_{\text{DAY}} = 118.44$, passive wakefulness: $F_{\text{DAY}} = 19.62$, light slow-wave sleep: $F_{\text{DAY}} = 67.88$, deep slow-wave sleep: $F_{\text{DAY}} = 99.06$, REM sleep: $F_{\text{DAY}} = 23.91$, number of SWDs: $F_{\text{DAY}} = 10.08$, all $df = 9, 63$, all $P < .05$. Immediately after the phase shift all acrophases were advanced of 43.00–151.87 degrees, except the acrophase of the passive wakefulness. Phase-shift magnitude (the difference between the position of acrophase between the baseline and the 1st phase-shift day) was different for the different sleep–wake states (one-way ANOVA, $F = 23.50$, $df = 4, 35$; $P < .05$). The acrophase of REM sleep was affected the most, however, it accomplished the largest daily shifts and was the fastest to re-synchronize (4th post-shift day). Light slow-wave sleep and SWDs reached the baseline values on the 7th day after the shift. Active wakefulness and deep slow-wave sleep returned to the baseline on the 8th post-shift day. An overview of the re-synchronization is presented in Fig. 4.

During the re-synchronization process, circadian rhythmicity in active wakefulness and both slow-wave sleep stages, determined by a significant fit of a cosine to the data, was present in all animals, during all experimental days after the phase-shift. However, a cosine was less well fitted for the number of SWDs, passive wakefulness, and REM sleep and this demonstrates that these rhythms showed a poorer temporal organization. These two latter states showed the greatest abnormalities during the course of re-synchronization, even on the 10th post-shift day, the expression of passive wakefulness and REM sleep in some rats was not rhythmic as yet. On the first post-shift day a large peak of the REM sleep emerged during the dark phase changing

the shape of the rhythm into bimodal. The peak persisted till 8th post-shift day, as judged by a visual inspection of a double-plotted actogram (Fig. 5). Contrary to the REM peaks, the light–dark transition-related minimum did not change its position during re-synchronization (Friedman ANOVA, $F = 21.82$, $df = 9$, $P < .05$, Dunn's post-hoc test, baseline vs post-shift days, all $P > .05$). Considering passive wakefulness, significant difference in maximal peak position was seen only on the 4th post-shift day (Friedman ANOVA, $F = 19.62$, $df = 9$, $P < .05$, Dunn's post-hoc test baseline vs post-shift days, $P < .05$), while the minimum of the rhythm, typically located at the transition between the dark and light phases, was significantly advanced into the dark phase for 4 post-shift days (Friedman ANOVA, $F = 39.16$, $df = 9$, $P < .05$, Dunn's post-hoc test, baseline vs post-shift days, all $P < .05$).

To verify whether particular rhythms were related to each other, time series of their duration for all experimental days were created (resolution: 6 min) and correlated with one another. The strength of the relationship given by an absolute value of Pearson's correlation coefficient (r) was compared by means of one-way ANOVA and was found significantly different between various pairs of rhythms ($F = 129.35$, $df = 14$, $P < .05$). The strongest relationship was seen for active-wakefulness–deep slow-wave sleep and active wakefulness–light slow-wave sleep pairs ($r = 0.76 \pm 0.01$ and 0.69 ± 0.01 , respectively, all $P < .05$, active-wakefulness–deep slow-wave sleep and active wakefulness–light slow-wave sleep vs the other pairs). The second was found for passive wakefulness and deep slow-wave sleep (0.56 ± 0.01 , all $P < .05$, passive wakefulness–deep slow-wave sleep relationship vs others investigated). The third were light–deep slow-wave sleep and active wakefulness–REM sleep pairs (0.42 ± 0.01 and 0.37 ± 0.01 , respectively; all $P < .05$, light–deep slow-wave sleep, active wakefulness–REM sleep pairs vs the remaining ones). The weakest relationships were found between REM sleep and the other sleep–wake states with an exception of active wakefulness (REM sleep–passive wakefulness: $r = 0.16 \pm 0.03$; REM sleep–light slow-wave sleep: $r = 0.07 \pm 0.02$; REM sleep–deep slow-wave sleep: $r = 0.10 \pm 0.01$; REM sleep–SWDs: $r = 0.07 \pm 0.01$). SWDs were correlated the most with deep slow-wave sleep ($r = 0.25 \pm 0.02$), however the strength of the mutual relationship did not differ significantly from those with active wakefulness and light slow-wave sleep ($r = 0.21 \pm 0.02$, 0.16 ± 0.02 , respectively, all $P > .05$). Data are presented in Fig. 6.

DISCUSSION

Re-synchronization of various sleep–wake states and absence seizures to an 8 h light phase delay was investigated in a validated animal model of childhood absence

Fig. 3. Total daily duration (minutes) of sleep–wake states (mean $\pm$ SEM, $n = 8$), occurrence (number) and mean duration (seconds) of SWDs (mean $\pm$ SEM, $n = 8$) during baseline and post-shift days (A). Total duration of several sleep–wake states (mean $\pm$ SEM, $n = 8$), occurrence (number) and mean duration (seconds) of SWDs (mean $\pm$ SEM, $n = 8$) in the light and the dark phase of the photoperiod during baseline and post-shift days (B). * $P < .05$, baseline vs post-shift day, ANOVA for repeated measures, Bonferroni post-hoc test.

Fig. 4. Re-entrainment of sleep–wake states (mean $\pm$ SEM, $n = 8$) and SWDs (mean $\pm$ SEM, $n = 8$) after an 8 h light phase delay. Total duration of sleep–wake states in minutes and the number of SWDs during particular post-shift days were plotted with respect to the baseline. The dark phase of the 12:12 light–dark cycle is marked by gray rectangles. * $P < .05$, acrophase of the baseline vs acrophase on the post-shift day, # $P < .05$, nadir of the baseline vs nadir of the post-shift day, ANOVA for repeated measures, Bonferroni post-hoc test or Friedman ANOVA, Dunn's multiple comparison post-hoc test.

epilepsy, rats of the WAG/Rij strain. The present study firstly aimed to determine the time dynamics of the process and a putative relationship between vigilance rhythms and the rhythm of SWDs. Secondly, the effects of agomelatine were

evaluated with respect to duration of sleep–wake states and number of SWDs during stable entrainment (acute study) and after the phase shift (sub-chronic study). Different speeds of re-synchronization of sleep–wake states and

Fig. 5. Double-plotted actograms of the duration (minutes) of the different sleep–wake states (mean $\pm$ SEM, $n = 8$) and the number of SWDs (mean $\pm$ SEM, $n = 8$) across all experimental days. Each row represents 2 consecutive days of the experiment (48 h). The second day (24 h) in each row is then plotted again as the first day in the row below as marked by the thin arrows on active wakefulness actogram. The beginning of the shifted photoperiod (the first post-shift day) is marked by thick arrows. The dark phase of the 12:12 light–dark cycle is marked by gray rectangles. * $P < .05$, acrophase of the baseline vs acrophase on the post-shift day, ANOVA for repeated measures, Bonferroni post-hoc test or Friedman ANOVA, Dunn's multiple comparison post hoc test.

SWDs rhythms, correlations between various rhythms, and a lack of effect of agomelatine both in the acute and sub-chronic administration comprise the main findings.

Desynchronization between different sleep–wake states after a phase shift has been described previously in rats (Sei et al., 1992, 1994). Striking was the increase in REM

sleep duration during the dark phase. Considering shift magnitude and time differences in reaching baseline values, the acrophase of REM sleep was shifted the most, however, it re-synchronized as first. Although REM sleep propensity is well known to be controlled by the circadian timing system and inversely coupled to the body temperature rhythm in

Fig. 6. The strength of the mutual relationships (the absolute value of Pearson's correlation coefficient (r)) between various pairs of sleep–wake states and SWDs. * $P < .05$, one-way ANOVA, Bonferroni post-hoc test. Numbers above each data bar represents significant difference between that pair of sleep–wake states and the other pair coded by this particular number. AW = active wakefulness, PW = passive wakefulness, SWS L = light slow-wave sleep, SWS D = deep slow-wave sleep, REM = REM sleep, SWDs = spike-wave discharges.

humans (Czeisler et al., 1980) and rats (Cambras et al., 2007), the amplitude of the REM sleep rhythm is small in comparison to active wakefulness or slow-wave sleep (Borbély et al., 1975). Rhythms with small circadian amplitude accomplish larger shifts and their rate of re-synchronization is faster (Reinberg et al., 1978). In agreement with this theory, the rhythms of light slow-wave sleep and SWDs, both with average amplitude, completed the shift three days after REM sleep, while active wakefulness and deep slow-wave sleep, both have a large circadian amplitude, were the slowest to re-synchronize. However, it should be mentioned that although the acrophase of the REM sleep re-synchronized firstly, the increase in its daily amount, especially during the dark phase, was the most prolonged. Similar changes regarding duration of REM sleep were observed in mice subjected to chronic environmental circadian disruption (Brager et al., 2013).

Internal desynchronization is thought to be a consequence of desynchrony between anatomically and functionally distinct subregions of the suprachiasmatic nucleus (SCN). Circadian regulation of sleep, slow-wave sleep and locomotor activity was associated with the activity of both the ventrolateral and dorsomedial SCN, while circadian regulation of REM sleep depended merely on the activity of the dorsomedial SCN (Cambras et al., 2007; Lee et al., 2009). In the present study, the strongest relationship across all experimental days was seen between active wakefulness and both stages of slow-wave sleep, it was significantly stronger than active

wakefulness–REM sleep relationship. Moreover, REM sleep showed the weakest correlations with the remaining sleep–wake states and SWDs. Based on this finding, together with same re-synchronization rate, we hypothesize that active wakefulness and deep slow-wave sleep are governed by the same circadian mechanism distinct from REM sleep. Considering the speed of re-synchronization, the rhythm of SWDs and light slow-wave sleep re-entrained together which may suggest that both rhythms are controlled by a common mechanism.

The phase shift affected the timing of epileptic activity: while the total number of SWDs remained unchanged, a light–dark redistribution occurred. After the shift, SWDs were more prevalent during the light and less prevalent during the dark period. The elevation during the light was accompanied by an increase in the total duration of passive wakefulness and a marked reduction of deep slow-wave sleep. Considering the tight relationship between SWDs and vigilance (Drinkenburg et al., 1991), it seems that the balance between SWDs enhancing and inhibiting brain states is shifted towards favorable conditions for SWDs to occur. Similar results were obtained in our earlier study, in which WAG/Rij rats were exposed to the same light–dark manipulation, however, without pharmacological intervention and without an explanation in terms of sleep–wake states (Smyk et al., 2012).

Neither dose-dependent nor drug effects of agomelatine on sleep–wake states and SWDs were found in the acute study. However, an ‘hour $\times$ day’ interaction was noticed for the number of SWDs; they were decreased for four consecutive hours during the treatment day. It cannot be excluded that Cyclodextrin (20%) used as a vehicle and given only on treatment day accounted for the effect observed. Cyclodextrins are cyclic oligosaccharides containing 6–8 glucose units connected by α -(1, 4) bonds. This particular formation (hydrophilic outside, hydrophobic inside) accounts for their ability to create inclusion compounds with other hydrophobic molecules increasing their stability, solubility and bioavailability and are widely used (Duchene et al., 1986; Davis and Brewster, 2004). Considered as neutral solvent, cyclodextrins were reported to exert neuroactive effect both in vitro and in vivo (Wang et al., 2003; Shu et al., 2004; Pytel et al., 2006). In WAG/Rij rats, hippocampal microinjections of cyclodextrin (45% solution) had a biological relevant effect (it reduced the number of SWDs to 1 h after the administration) (Tolmacheva and van Luitelaar, 2007). In the present study, the number of SWDs was decreased during the treatment day between 4 and 8 h after administration. Differences in time, during which the effect was manifested may be attributed either to the route of administration: local vs systemic or, considering a slight passage of cyclodextrin through blood–brain barrier (Monnaert et al., 2004), behavioral effects of the administration such as arousal, stomach load, increased thirst may be responsible for the effect observed. Also, in the sub-chronic study the daily oral administration of agomelatine did not affect any of the characteristics of the rhythms investigated, nor the speed of the re-synchronization after the phase shift. Given the chronobiological character of the study, dosage of the compound for both experiments

(between 2.5–10 mg/kg), was based on its ability to synchronize circadian rhythms, as it was shown previously that an oral dose of 5.7 mg/kg provided true entrainment (Martinet et al., 1996). Moreover, the highest dose used in the present study (10 mg/kg) was found to induce changes in sleep–wake architecture in a period between 4 and 7 h after the administration and also during the following day (Descamps et al., 2009). The reasons behind the lack of the agomelatine effectiveness observed in the present study might be as follows: both experiments of the present study were conducted in 12:12 light–dark cycle in contrast to constant darkness as in Martinet et al. (1996), therefore, it cannot be excluded that the presence of this powerful Zeitgeber outweighed the effects of the compound. Furthermore, the transient arousal (Hastings et al., 1992) produced by oral administration of the drug might have masked its effect on the rhythms. On the other hand, our results are in accordance with other studies concluding that agomelatine, when given shortly before the dark onset, failed to affect sleep–wake states in nocturnal rodents (Tobler et al., 1994).

To conclude, the present study demonstrates internal desynchronization between various sleep–wake states and SWDs after a rapid shift in the timing of the light phase of the photoperiod. The SWDs rhythm re-entrained together with light slow-wave sleep, a state frequently preceding the occurrence of absence seizures. This suggests a common mechanism for their co-occurrence, distinct from the one governing activity and deep slow-wave sleep. Circadian misalignment caused by the phase shift resulted in an aggravation of epileptic activity in the light phase, during which SWDs in WAG/Rij rats are more sparse than in the dark phase. An increased duration of passive wakefulness seems to be responsible for the increase, since, this sleep–wake state acts as SWDs enhancer. Treatment with the melatonin agonist was found ineffective in the acute sleep–wake study, as well in the phase delay paradigm. It also lacked effect on epileptic activity. The redistribution of the sleep–wake states across the 24 h during the re-entrainment process might be of significant importance for epilepsy patients, who are planning trans meridian flights across time zones and are dealing with adverse consequences of jet lag.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiment: MKS, GvL, HH, WHD. Performed the experiments: MKS, HH. Analyzed the data: MKS, GvL. Wrote the paper: MKS, GvL, HH, WHD.

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