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Periodic limb movements in sleep exhibit a circadian rhythm that is maximal in the late evening/early night

Jeanne F. Duffy, MBA, PhD^{*}, Alex S.W. Lowe, Edward J. Silva, and John W. Winkelman, MD, PhD

Division of Sleep Medicine, Department of Medicine, Brigham and Women's Hospital and Division of Sleep Medicine, Harvard Medical School Boston, MA 02115 USA

Abstract

Objective—Periodic limb movements in sleep (PLMS) show a time-of-night pattern, with most movements at the beginning of the night. Our study aimed to determine whether this pattern is due to an endogenous circadian rhythm, like that in the related movement disorder Restless Legs Syndrome (RLS).

Methods—Four healthy older adults with a screening PLMI > 20 were studied in an inpatient forced desynchrony protocol with an imposed sleep-wake cycle of 20 hours for twelve “nights,” allowing separation of circadian and sleep homeostatic influences on leg movements. We recorded sleep polysomnographically throughout each scheduled episode, including left and right anterior tibialis EMG.

Results—PLMS in Stage 2 showed both a significant time-within-sleep pattern and a significant circadian rhythm. The circadian rhythm in PLMS peaked at the circadian phases when usual sleep onset occurs, preceding the evening rise in melatonin secretion.

Conclusions—In our subjects, the circadian pattern of PLMs expression was very similar to that previously reported in patients with RLS. This evidence for a circadian rhythm in PLMS has implications for treatment and provides direction for future studies of the pathophysiology of this movement disorder.

Suggested key words

circadian rhythm; sleep; melatonin; movement disorder; RLS; PLMS

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^{*}to whom correspondence should be addressed: 221 Longwood Avenue, BLI 438, Boston, MA 02115-5817 USA, Tel: 617-732-4013, Fax: 617-732-4015, jduffy@hms.harvard.edu.

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Conflicts of interest.

This is not an industry-supported study and did not test any investigational drug or device. Mr. Silva and Mr. Lowe report no conflicts of interest. Dr. Duffy reports that she has received research grants from Philips-Respironics, Inc. that are unrelated to the present work. Dr. Winkelman reports that he has received research support from GlaxoSmithKline and Sepracor; he is a member of the speakers bureau for Sanofi-Aventis and Sepracor; he is a consultant or member of an advisory board for Covance, GlaxoSmithKline, Impax Laboratories, Luitpold Pharmaceuticals, Neurogen, Pfizer, and Zeo.

Introduction

Periodic Limb Movements in Sleep (PLMS) are characterized by repetitive, periodic (every 15–45 seconds) flexions of the extremities (predominantly the legs) during sleep, with each movement lasting 0.5–5 seconds [1]. The majority of these movements occur in the anterior tibialis and gastrocnemius muscles [2] and are associated with sympathetic nervous system activation [3] and arousals from sleep [4]. PLMS are present in roughly 50% of individuals over 65 years of age [5,6], and are also common in a variety of sleep and neurological disorders that are characterized by dopaminergic dysfunction (e.g., Restless Legs Syndrome, Narcolepsy, REM-Behavior Disorder; see [7] for review). PLMS in a patient with insomnia or hypersomnia without other known causes can be diagnosed as Periodic Limb Movement Disorder (PLMD) [1].

Most research on PLMS has been performed in patients with Restless Legs Syndrome (RLS), a sensory-motor disorder characterized by leg dysesthesia and restlessness while awake and inactive [8]. RLS affects 5–10% of the adult population [9–11], and roughly 80% of RLS patients also have PLMS [4]. One of the defining features of RLS is its circadian rhythm, with increased symptom severity in the evening and night compared with the daytime [8]. The endogenous nature of this 24-hour pattern of RLS severity has been established by keeping patients awake and restricted to bed rest while their symptoms are periodically assessed throughout day and night [12].

Two studies using overnight polysomnography have demonstrated a time-of-night pattern in PLMS leg movements for many patients, with the movements occurring predominantly in the first three hours of the night (Type I pattern [13,14]), with a stable pattern within an individual patient over two nights of study. In the majority of patients with this Type I pattern, rates of PLMS in the first two hours of sleep were over five times those seen in the last three hours of sleep, even when adjusted for time-of-night differences in rapid eye movement (REM) sleep [4]. Whether this represents an underlying circadian rhythmicity in PLMS, as in RLS, or instead is a consequence of the high homeostatic sleep pressure associated with the beginning of nocturnal sleep episode, is unclear. Distinguishing between these two possibilities (a circadian rhythm or a homeostatic sleep-dependent process underlying the movements), and/or determining the relative contributions of these two processes, will provide insight into the development of new treatments and the best use of existing treatments.

The most powerful tool to separate and quantify independent circadian and homeostatic influences is forced desynchrony protocol [15,16]. During this protocol, subjects are scheduled to a rest-activity schedule which is outside of the range of entrainment of the biological clock (e.g., several hours shorter or longer than 24 hours), allowing the circadian system to cycle at its near-24 hour intrinsic periodicity. The protocol results in sleep and wake episodes that are distributed across the circadian cycle, with a similar duration of wakefulness prior to each sleep episode. By using appropriate analysis techniques, the influence of circadian phase can be separated from the influence of homeostatic sleep pressure, allowing evaluation of their independent influences on the data. We therefore used forced desynchrony protocol to assess the circadian and homeostatic influences on the pattern of PLMS in the present study.

Methods

Ethical approval

The study was approved by the Partners HealthCare Human Subjects Committee and was conducted in accordance with the Declaration of Helsinki. Each subject gave written informed consent prior to study.

Subjects and screening

Subjects were recruited from newspaper advertisements and flyers posted in the community for a month-long inpatient study. They had to be 55 years of age or older, free from acute and chronic medical problems, not taking medications, and without significant complaints about their sleep or reports of daytime sleepiness (Epworth Sleepiness Scale <10 [17]). They were screened to verify that they were medically (serum chemistry, complete blood count, urinalysis, electrocardiogram, physical examination) and psychologically (Geriatric Depression Scale, Minnesota Multiphasic Personality Inventory, Mattis Dementia Rating Scale, Folstein Mini-Mental State Exam, and clinical interview) healthy. As part of their screening, they had an all-night polysomnographic study to rule out clinically significant sleep disordered breathing (using oximetry, respiratory effort, and a nasal pressure transducer) and to determine whether they had periodic leg movements during sleep. Of a total 25 subjects who met all the screening criteria for the main study (on the effects of pre-sleep melatonin administration on sleep), four subjects (1 woman, 3 men) had a screening PLMI > 20 and were included in the analysis reported here. See Table 1 for subject demographic and screening information.

Subjects were questioned about RLS-like symptoms via three questions on pre-study questionnaires. Three of the subjects answered “never” or “rarely” to all three questions. The fourth subject answered “never” or “rarely” to two of the questions, but “often” to the final question. All four subjects indicated that they did not have a family member with RLS on the questionnaire. See Table 2 for the text of these screening questions.

Study conditions

During the study, each subject lived in a private room without information about time of day in the Intensive Physiological Monitoring Unit at the Brigham and Women’s Hospital General Clinical Research Center. Room temperature was kept at approximately 24°C, and ambient light levels during wake episodes were controlled by the experimenters and were kept dim [$<0.0087 \text{ W/m}^2$ (~3.3 lux) at 137 cm from the floor facing the walls, maximum of 0.048 W/m^2 (15 lux) at 187 cm from the floor facing the ceiling]. During scheduled sleep episodes, the lights were turned off. The subjects were allowed to shower after each wake time, received three regular meals each wake episode, and took computer-administered performance test batteries lasting ~20 minutes at 2-hour intervals and shorter alertness assessments every half hour. Approximately 2 hours before each scheduled bedtime, a study technician began applying electrodes to the subject’s face and scalp to conduct the polysomnographic sleep recording (see below), a procedure that took approximately an hour. Approximately one hour before each scheduled bedtime, the subject got into his/her bed, where he/she remained sitting with the head of the bed raised until just prior to scheduled lights out. When they were not scheduled to do study events, the subjects were free to pursue sedentary activities within their study room, but they were not allowed to sleep, lie down, or nap during scheduled wake episodes.

Each study began with three baseline nights of 8 hours time in bed, scheduled at each subject’s habitual times recorded at home the week prior to the study. Following this, each subject was scheduled to live on a 20-hour rest-activity cycle for 30 cycles (26 calendar

days), with two-thirds of each “day” (13.33 hours) spent awake and one-third (6.67 hours) spent lying in bed in the dark attempting to sleep (Figure 1). This forced desynchrony (FD) protocol was designed to remove the synchronizing influence of the rest-activity cycle on the circadian pacemaker, allowing the circadian pacemaker to free-run at its endogenous, near-24-hour cycle length. On the first 12 cycles of forced desynchrony the subjects received a capsule containing melatonin 30 minutes before lights out, for the following 6 nights they received capsules containing placebo (a “washout” study segment), and for the remaining 12 cycles of forced desynchrony they received a placebo capsule prior to lights out. We include here only the final 12 forced desynchrony nights where the subjects received placebo.

Data collection

Sleep was recorded polysomnographically for each scheduled episode using a standard montage that included electro-encephalographic (EEG) signals (C3, C4, O1, O2) referenced to contralateral mastoids (A1, A2), two electrooculograms (EOG; left outer canthus, right outer canthus), one submental electromyogram (EMG), and a 2-lead electrocardiogram. In addition, left and right anterior tibialis EMG signals were recorded to detect PLMS. All signals were acquired using a digital ambulatory sleep recording system (Vitaport-2 or 3, Temec Instruments, Kerkraade, B.V., The Netherlands). The EEG signals were high-pass filtered at a time constant of 1 second and low-pass filtered at 70 Hz. Finally, the signals were digitized with a resolution of 12 bit (range 500 μ V; sampling rate 256 Hz, storage rate 128 Hz), stored on a Flash RAM card, and downloaded offline after wake time. Following the study, all sleep recordings were staged in 30-second epochs by a trained technologist according to established criteria [18]. Leg movements were scored by one of us (A.S.W.L.) according to established criteria [19]. Movements for each leg, as well as bilateral movements, were scored separately.

Circadian period for the segment of the study reported here was assessed using core body temperature data collected at 1-minute intervals using a rectal thermistor (Measurement Specialties, Inc., Hampton VA). Non-orthogonal spectral analysis (NOSA [16]) was used to assess circadian period. This method takes into account the influence of the imposed 20-hour rest-activity cycle and then searches for an unknown periodicity within the circadian range (search range used in this study was 15–30 hours), using an exact maximum-likelihood fitting procedure [20]. This analysis also provided the time of the circadian temperature nadir at the beginning of the study segment (set equivalent to 0 circadian degrees), and this, combined with the circadian period, allowed us to assign a circadian phase (from 0° to 359°) to each 30-second epoch of sleep and to each melatonin sample. We binned the data into 60° circadian phase bins (equivalent to ~4 hours) for further analysis. We also assigned a time within sleep to each 30-second epoch of scheduled sleep, and then binned the data into hourly time within sleep bins for further analysis.

Because stage 2 sleep is the most abundant sleep stage, PLMS are most likely to occur during stage 2, and because there is minimal circadian variation in the occurrence of stage 2, we conducted our PLMS occurrence analysis with respect to circadian phase or time within sleep restricting our analysis to stage 2. This analysis was conducted using generalized linear mixed model analysis (SAS 9.1; SAS Institute, Cary, NC) on raw data sets and assumed a Poisson distribution. Main effects of circadian phase and time within sleep were assessed by incorporating into the model a random intercept statement allowing for means to vary between subjects. All effects were treated as categorical rather than continuous variables.

Blood samples were collected approximately every 60 minutes throughout most of the study using an indwelling venous catheter. The resulting plasma was frozen and assayed for

melatonin after the study in either the Brigham & Women's Hospital General Clinical Research Center Core Laboratory or at Pharmasan Labs (Osceola, WI). We assigned a circadian phase to each melatonin sample as described above.

For illustration of PLMS and melatonin data, we binned the data with respect to circadian phase or time within sleep for each subject and then averaged across subjects.

For all statistical tests, the critical significance level was defined as $\alpha = 0.05$. Unless otherwise indicated, results are reported as mean $\pm$ standard deviation.

Results

Baseline nights

Total sleep time averaged 362.9 ± 32.4 minutes on the three 8-hour baseline nights (range 307.5 – 404 minutes). Sleep efficiency (defined as the percentage of time between lights off and lights on spent in any sleep stage) on the baseline nights therefore averaged $75.5 \pm 6.3\%$. The average PLMI on the baseline nights was 53.2 ± 38.1 (range 7.6–99.6). Subjects averaged 191 ± 37.8 min of stage 2 sleep each night (range 147–274 min), representing $52.5 \pm 9.4\%$ of their total sleep time (range 38.2–67.9%). We found a significant effect of time within sleep on PLMS ($F_{7,4574} = 39.51$, $p < 0.0001$), with the greatest number of PLMS occurring in the first half of the scheduled sleep episode (Figure 2, upper panel).

Forced desynchrony nights (FD; sleep at different circadian phases)

Two nights (one each from two subjects) were not included in our analysis due to technical problems with the recording causing missing data. A total of 46 FD nights were included. Total sleep time on the 6.7-hour FD nights averaged 258.6 ± 78.6 minutes (range 115–374 minutes). Sleep efficiency on the FD nights averaged $67.4 \pm 18.5\%$. Subjects averaged 127.1 ± 49.2 min of stage 2 sleep each night (range 37–221.5 min), representing $32.8 \pm 11.7\%$ of their total sleep time (range 9.5–55.4%) and totaling 11,693 epochs of stage 2. We found a significant effect of time within sleep on the occurrence of PLMS ($F_{6, 11678} = 77.97$, $p < 0.0001$), with the greatest number of stage 2 PLMS occurring in the first 4 hours of the 6.67-hour scheduled sleep episode (Figure 2, lower panel). We also found a significant effect of circadian phase on the occurrence of PLMS during stage 2 sleep ($F_{5, 11678} = 49.28$, $p < 0.0001$). The fewest PLMS occurred in epochs of stage 2 sleep that fell in the range of circadian phases between 0 – 180° , and the greatest number of PLMS occurred in epochs of stage 2 sleep that fell in the circadian phases in the range of 180 – 360° (Figure 3, lower panel). The peak in the rhythm of PLMS occurred at the circadian phase bins centered at 240° and 300° , just before the circadian phase bins when the peak in endogenous melatonin secretion occurred (300° and 0° ; Figure 3, upper panel). This time of peak PLMS frequency occurs at the circadian phases corresponding to just before habitual bed time under normal entrained conditions.

Discussion

Previous studies of PLMS have been conducted on sleep scheduled at night, when the subjects/patients normally sleep, and have consistently demonstrated that PLMS occur predominantly at the beginning of the sleep episode [14], with a reproducible pattern from night-to-night within an individual. Our current forced desynchrony data reveal that this pattern is so prominent because, in individuals sleeping at night, both circadian and homeostatic influences independently predispose PLMS to be expressed at this time.

Symptoms of the related movement disorder RLS are also most prominent in the evening and near the time of the beginning of the habitual sleep episode, a finding that has been

documented using a modified constant routine protocol in which subjects were kept awake throughout day and night to monitor their RLS symptoms [12,21,22]. Our current demonstration that the circadian predisposition of PLMS is nearly identical to that of RLS (with maximal expression at the circadian phases corresponding to the late evening, and maximal expression early in the sleep episode) further reinforces our understanding of the similarities between these two movement disorders: PLMS are present in roughly 80% of individuals with RLS [4], treatment of PLMS can produce RLS in previously asymptomatic individuals [23], the known single nucleotide polymorphisms in individuals with RLS strongly code for PLMS [24], and dopaminergic agents can dramatically suppress both PLMS and RLS [25].

In a previous study employing a constant routine protocol in patients with RLS, Michaud et al. theorized that circadian fluctuations in CNS melatonin levels account for the circadian pattern of RLS symptoms [12]. They reported that melatonin, body temperature, and vigilance all covaried with RLS discomfort and PLMs while awake, and that the onset of the melatonin rhythm preceded the acrophase of RLS symptom severity. Our data from non-RLS subjects with PLMs are in contrast with their finding, as we found that melatonin onset occurred *after* the peak of PLMS activity. However, our finding does not preclude the possibility that there are separate influences on the sensory symptoms of RLS and the sleep-related motor sign, PLMS, particularly given that our study was not performed in individuals with RLS.

Future studies using the forced desynchrony protocol in RLS patients should be conducted to assess the independent roles of circadian and homeostatic influences on RLS symptom severity. Studies that include patients with PLMs associated with RLS, or in patients with PLMs associated with other causes, would further define whether such a circadian pattern is an underlying feature in all individuals with nocturnal leg movements. In addition, analysis of genetic material from such study participants to examine genes associated with circadian rhythms and/or movement disorders could be done simultaneously. Such information could then inform treatment: if there are different subtypes of PLMs characterized by the presence or absence of a circadian rhythm, the subtypes may differentially respond to different treatments. It is also possible that the circadian pattern of PLMs differs between patient groups, and in such cases the timing of a treatment could be optimized.

In addition, larger studies to identify the underlying cause of the circadian variation in PLMS can be conducted now that there exists clear evidence of a sleep-independent circadian rhythm to such movements. Such future studies should include forced desynchrony studies in PLMS patients in which potential output signals from the biological clock and/or endocrine rhythms [26,27] that might contribute to PLMs are measured simultaneously with leg movements. In particular, forced desynchrony studies in which dopamine metabolites are measured across the circadian cycle and throughout sleep episodes should be done. PLMS is currently understood as a disorder of dopaminergic dysfunction, due to the dramatic reductions in these movements with dopaminergic precursors or agonists [28,29], the worsening of PLMs with dopaminergic antagonists [30,31], and the presence of PLMs in disorders associated with hypodopaminergic function [32]. There are reports from animal studies that dopamine receptor binding [33;34] and dopamine content [35,36] in various brain regions show a bimodal pattern, with peaks approximately midway through both the light and dark phases. In humans, the dopaminergic metabolite homovanillic acid in plasma (pHVA) has become a standard measure to assess dopamine levels in the CNS [37–39]. A well-controlled study by Sack et al. assessed pHVA levels at 2-hour intervals across 28 hours in constant conditions (awake, with controlled posture, activity, and food intake) in healthy young adults [40]. They reported that pHVA levels were higher during the night than the day, with peak values in the middle of the night and minimum values shortly

after habitual wake time. Thus, a circadian variation in dopamine levels could contribute to the expression of PLMs, and a forced desynchrony study in which circadian phase and sleep are systematically varied while measuring pHVA and PLMs will allow better understanding of the role of dopamine in PLMs expression. Such studies should include careful assessment of iron status and evaluation of symptoms prior to and following each sleep episode. While the present analysis has certain limitations, given that it was conducted in a small number of subjects in whom iron status was not documented, our findings are important in establishing that PLMs in non-RLS patients exhibit a circadian rhythm in their occurrence. Our findings also demonstrate that in this group of subjects, the peak in the circadian rhythm of PLMs precedes that of melatonin secretion, suggesting that alternative endogenous factors underlie the rhythmicity of leg movements in individuals with PLMs.

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Figure 1.

Raster plot of study protocol. The x-axis shows time of day and is 24 hours in length. The y-axis shows the day of the experiment, with each day shown beneath the previous day. Black bars indicate scheduled sleep episodes. The first three 24-hour baseline days (8 hours scheduled bedrest) were scheduled at the subjects' habitual times. Following this was the forced desynchrony segment, during which the subjects were scheduled to live on a 20-hour rest-activity cycle for 30 cycles (26 calendar days), with one-third (6.67 hours) of each "day" spent lying in bed in the dark attempting to sleep. The final three 24-hour recovery days were scheduled at the same times as the baseline. Non-shaded areas on the plot indicate those study segments that were included in our analysis.

Figure 2.

Pattern of periodic limb movements of sleep (PLMS) across the night during baseline nights (upper panel) when sleep was scheduled for 8 hours at habitual times, and during forced desynchrony (lower panel) when sleep was scheduled for 6.67 hours at many circadian phases. Mean $\pm$ standard deviation PLMS per hour of stage 2 sleep are plotted with respect to elapsed time since lights out. Data were binned per subject and then averaged across subjects.

Figure 3.

Pattern of endogenous melatonin secretion (upper panel) and periodic limb movements of sleep (lower panel) with respect to circadian phase during forced desynchrony. Upper panel: Plasma melatonin data from each subject were averaged per 60° circadian phase bin (~4 hours), and then averaged across the four subjects. Mean $\pm$ standard deviation are double-plotted with respect to the center of the bin. Lower panel: PLMS counts per hour of stage 2 sleep from each subject were averaged per 60° circadian phase bin, and then averaged across the four subjects. Mean $\pm$ standard deviation are double-plotted with respect to the center of the bin. The upper x-axis indicates corresponding time of day under entrained conditions. The shaded area indicates where the nightly sleep episode would occur, on average, during entrained conditions.

Table 1

Subject information.

	Mean $\pm$ SD	Range
Age	63.3 $\pm$ 10.4	56 – 78
Screening PLMI	58.3 $\pm$ 32	28 – 103
Epworth Sleepiness Scale Score	3.25 $\pm$ 2.22	0 – 5
Habitual Bedtime	23:10 $\pm$ 0:15	22:55 – 23:32
Habitual Wake time	07:14 $\pm$ 0:16	07:00 – 07:34

Table 2

Screening questions related to RLS symptoms, and subject responses.

Screening Question	Subject Response			
	A	B	D	C
My sleep is disturbed by 'restless legs' (a feeling of crawling, aching, inability to keep legs still).	rarely	never	never	not sure
Which of the following do you notice when you try to fall asleep? Need to move legs.	often	never	never	rarely
Which of the following do you notice when you try to fall asleep? Twitches in hands, feet arms, legs.	rarely	never	rarely	rarely
Some family member has 'restless legs' while sleeping (a feeling of crawling, aching, inability to keep legs still).	never	never	never	rarely