

# Influence of sleep stage and wakefulness on spectral EEG activity and heart rate variations around periodic leg movements

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Accepted 27 April 2004

Available online 7 June 2004

## Abstract

**Objective:** Typical changes in spectral electroencephalographic (EEG) activity and heart rate (HR) have been described in periodic leg movements (PLM) associated with or without microarousals (MA). We aimed to determine the effects of sleep stage and wakefulness on these responses to ascertain whether a common pattern of EEG and HR activation takes place.

**Methods:** The time course of EEG spectral activity and HR variability associated with PLM was analysed in 13 patients during light NREM sleep, rapid-eye-movement (REM) sleep and wakefulness. The same analysis was also conducted for PLM without MA occurring in stage 2.

**Results:** A significant EEG and electrocardiogram (ECG) activation was found associated with PLM during sleep, but not during wakefulness. While in light NREM sleep, an increase in delta and theta bands was detected before the PLM onset, in REM sleep the EEG activation occurred simultaneously with the PLM onset. Moreover, during stage 1 and REM sleep, alpha and fast frequencies tended to remain sustained after the PLM onset. In contrast, during wakefulness, a small and not significant increase in cerebral activity was present, starting at the PLM onset and persisting in the post-movement period. A typical pattern of cardiac response was present during NREM and REM sleep, the autonomic activation being lesser and prolonged during wakefulness.

**Conclusions:** We conclude that the EEG and HR responses to PLM differ between sleep stages and wakefulness with lesser changes found during wakefulness.

**Significance:** These findings suggest that specific sleep state-dependent mechanisms may underlie the occurrence of PLM.

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**Keywords:** Periodic leg movement; Wakefulness; Sleep; Sleep stage; Spectral electroencephalographic analysis; Heart rate

## 1. Introduction

Periodic leg movements (PLM) are short-lasting movements of the lower limbs occurring periodically every 20–40 s (Coleman, 1982) during sleep and wakefulness, and present in 86% of patients suffering from restless legs syndrome (RLS; Montplaisir et al., 1997), a condition characterized by leg paresthesia and a urge to move the legs (Ekbom, 1960). PLM during sleep (PLMS) can also be associated with other sleep-related disorders such as sleep apnea or narcolepsy (Montplaisir et al., 1994a) or they can

be present in patients presenting no evidence of any sleep disorder (Coleman et al., 1980).

In the majority of patients, PLMS are often associated with microarousals (MA), a short-lasting electroencephalographic (EEG) activation, considered as a consequence of the PLM arising from sleep and leading to an unrestorative sleep and complaints of daytime sleepiness (Rosenthal et al., 1984; Saskin et al., 1985). Despite occurrence of the PLMS seems to induce the appearance of the MA, PLMS may also occur without changes in the EEG activity (Pollmächer and Schulz, 1993) or they could be associated with bursts of slow EEG activity (Sforza et al., 1999), in all cases inducing a transient rise in blood pressure (Ali et al., 1991) and heart rate (HR; Sforza et al., 1999, 2002).

Almost all studies investigating the characteristics of PLM conducted so far were made during NREM sleep and the few studies investigating the effects of NREM,

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rapid-eye-movement (REM) and wake state used visual scoring (Montplaisir et al., 1985; Nicolas et al., 1999; Pollmächer and Schulz, 1993). The aim of the present study is to examine the time course of spectral EEG activity and HR variations around PLMS with and without MA and PLM arising from wakefulness (PLMW), in order to assess whether a common pattern of cerebral and cardiac activation is present and independent of sleep–wake occurrence. The use of these analyses would help us to draw a better picture of the effects of sleep and wake states on characteristics of PLM and to understand the mechanisms triggering the appearance of the motor event.

## 2. Methods

### 2.1. Patient population

Thirteen patients (8 men and 5 women, mean age:  $51.7 \pm 3.3$  years, range: 30–72 years; mean body mass index:  $25.5 \pm 1.4$ , range: 19.6–38.1) were included in the study. All patients fulfilled the clinical criteria for the diagnosis of RLS ( $n = 10$ ; Walters, 1995) or PLM disorder (PLMD;  $n = 3$ ; Coleman, 1982). Inclusion criteria were: (1) absence of sleep disturbances such as sleep apnea and narcolepsy; (2) absence of neurological, cardiac or psychiatric disorders; (3) no intake of drugs acting on vagal–sympathetic balance and/or affecting EEG activity; and (4) presence of PLM during light NREM sleep, REM sleep and wakefulness. The most frequent complaints in RLS patients were unrefreshing nocturnal sleep, fatigue and insomnia, with daytime sleepiness in 3 patients (mean Epworth Sleepiness Scale (ESS) score:  $10.3 \pm 1.7$ , range: 4–18). At the International Restless Legs Syndrome Study Group (IRLSSG) severity score (Walters, 1995), they reported moderate to severe symptoms with a mean score of  $28.1 \pm 2.3$ . PLMD patients complained of excessive daytime sleepiness (mean ESS score:  $18 \pm 2.2$ ) and their IRLSSG severity score was  $0.8 \pm 0.2$ . In these patients, polysomnography ruled out upper airway resistance syndrome. Patients were informed that some of the collected data would be used for research purposes and they gave written informed consent.

### 2.2. Nocturnal sleep studies

Patients underwent one or two nights of polysomnographic recording with time in bed scheduled between 22:00 and 07:00 h, which represented their average daily sleep period. The data of the 3 patients who slept two nights at the laboratory were averaged. Polysomnography was performed using 3 EEG leads (C3–A2, C4–A1, O2–A1), right and left electrooculogram, chin electromyogram (EMG) and electrocardiogram (ECG). In order to assess apneas and hypopneas, nasal and oral airflows were recorded with thermistors or a pressure transducer (Protech2, Minneapolis, MN, USA),

and thoracic and abdominal respiratory movements with strain gauges. Oxygen saturation (SaO<sub>2</sub>) was measured continuously with a finger oxymeter. None of the patients showed an index of respiratory events (AHI) greater than 10 per hour of sleep. Tibialis muscle EMG activity was monitored using surface electrodes placed on the lower third of the right and left legs. EMG signal was recorded at a time constant of 0.3 s and a high bandpass filter setting of 90 Hz. A 50  $\mu$ V sinusoidal calibration signal of approximately 1 min duration was obtained in all subjects at the start of monitoring.

Sleep was scored according to standard criteria using 20 s epochs (Rechtschaffen and Kales, 1968) and the following sleep parameters were defined: total sleep time, sleep latency, sleep efficiency (total recording time/total sleep time  $\times$  100), wake after sleep onset, number of awakenings and percentage of each sleep stage.

### 2.3. Data analysis

The procedure employed for the time-dependent analysis of EEG and ECG activity has been described previously (Sforza et al., 1999, 2000) and is outlined below.

#### 2.3.1. Arousal and PLM analysis

PLMS were scored using Coleman's criteria (Coleman, 1982), i.e. movements lasting 0.5–5 s with inter-movement intervals of 4–90 s and occurring in series of at least 4 consecutive movements. During wakefulness, the duration of PLMW was extended to 10 s according to standard criteria (Michaud et al., 2002). During sleep, two analyses were performed. The first one considered only PLMS associated with MA occurring in stages 1 and 2 of NREM sleep and in REM sleep, since only a reduced number of PLMS associated with MA can be observed during slow wave sleep (SWS; Pollmächer and Schulz, 1993; Sforza et al., 1999). The second analysis was restricted to PLMS without MA occurring in stage 2, since few PLMS without MA were detected in stage 1 and REM sleep. MA was defined, according to ASDA criteria (ASDA, 1992) as a return to alpha or fast frequency, well differentiated from the background EEG activity. The duration was, however, extended to include MA lasting  $>1.5$  and  $<3$  s (Martin et al., 1997). MA detection criteria for REM sleep included an increase in submental EMG amplitude, in addition to a shift in EEG activity. The PLMS was scored as associated with MA if the latter occurred simultaneously or within 1 s before or after the onset of tibialis muscle EMG activity (Karadeniz et al., 2000; Mendelson, 1996). To optimize the detection of the PLM onset, any increase in the amplitude of the EMG signal (even when the amplitude was less than 20%) was considered as the beginning of the motor phenomenon. Analysis was done for PLM separated by a minimum of 20 s, after rejection of PLM showing EEG and/or ECG artefacts. For each sleep stage, the mean duration of the considered PLM and MA were measured.

### 2.3.2. EEG spectral and heart rate analysis

The EEG signals were digitized at a sampling rate of 128 Hz with a low pass filter at 30 Hz. Power spectra were calculated with a commercial software package (Prana Phitools®, Grenoble, France) which computes fast Fourier transform that was performed on the C3 or C4 lead in all patients. The EEG power spectra were computed for 1-s non-overlapping windows using a Hanning window tapering. The absolute power values ( $\mu\text{V}^2/\text{Hz}$ ) of 6 EEG components were defined: delta (1–4 Hz), theta (4.5–7.5 Hz), alpha (8–11 Hz), sigma (11.5–15), beta1 (15.5–18 Hz) and beta2 (18.5–35 Hz) power. The spectral power was computed every second during 10 s prior to PLM onset and 10 s after.

To assess the HR changes around PLM, up to 10 heartbeats prior to PLM onset were analysed and event-related HR fluctuations were calculated during 10 heartbeats after PLM onset. QRS peaks were detected, and then the HR was calculated directly from the R–R interval. The power measured for each frequency band within each 1-s window and the measurements of HR were then normalized by expressing its value as a percent of the mean value before the PLM onset. This method was used in order to evaluate the magnitude of potential changes within a specific measurement over time, and to compare, albeit individual differences, the changes in activity from a sleep stage to another.

### 2.4. Statistical analyses

Comparison of sleep parameters and PLM index and duration during wakefulness and sleep was done using the Mann–Whitney test. One-way ANOVA for repeated measures with a Greenhouse–Geiser correction was used to evaluate the difference between sleep stages and wakefulness in terms of the duration of the PLM and the MA. Post hoc comparisons were performed using the Tuckey HSD-test. To assess any differences in the EEG and ECG activities among the 3 sleep stages and wakefulness, a two-way ANOVA for repeated measures with a Greenhouse–Geiser correction was computed with stage as the between factor and time (10 s before and 10 s after PLM onset) as the repeated measure. For comparisons that reached significance, post hoc analysis was performed using the Tuckey HSD-test. When a time effect was observed, a one-way ANOVA for multiple comparisons with a Bonferroni correction was done to assess the difference between stages at a given time. Differences were considered significant if they had values of  $P < 0.05$  after corrections.

All statistical analyses were performed with the Statistica statistical software package (Statistica for Windows, 6.0, StatSoft Inc, Tulsa, OK). Results in the text and in tables are presented as means  $\pm$  SEM.

## 3. Results

### 3.1. Polygraphic data

Table 1 summarizes the sleep and polygraphic variables among RLS and PLMD patients as well as polygraphic characteristics of PLM during total sleep and each sleep stage.

As expected, patients with RLS had a significant rise in sleep latency, wake after sleep onset and indices of sleep fragmentation. They also showed an increase in the amount of stage 1 and a reduction in sleep stage 2 and REM, reaching significance only for REM sleep. The average number of PLM was  $341.2 \pm 40.9$  and the average PLMS index was  $51.3 \pm 5.4$ . Overall, 62.2% of movements during sleep were associated with MA mostly occurring during stages 1 ( $22.0 \pm 1.9\%$ ), 2 ( $58.4 \pm 4.0\%$ ) and REM sleep ( $14.8 \pm 2.3\%$ ), and a very few ones occurring during stages 3 and 4 ( $0.66 \pm 0.3\%$ ). When we compared the indices of PLM index during wakefulness and sleep for RLS and PLMD patients (Table 1), there were no significant differences for PLM index during stage 1 and SWS, RLS

Table 1

Sleep parameters and periodic leg movement characteristics during wakefulness and sleep stages in patients with Restless Legs syndrome (RLS) and Periodic Limb Movement Disorder (PLMD) (mean  $\pm$  (SEM))

	RLS patients	PLMD patients	<i>P</i>
Total sleep time (min)	392.5 (3.2)	453.9 (6.7)	n.s.
Sleep latency (min)	39.0 (1.3)	19.7 (2.1)	0.04
Sleep efficiency (%)	76.4 (1.3)	81.9 (1.89)	n.s.
Wake after sleep onset (min)	125.1 (3.0)	66.6 (4.0)	0.03
Awakenings (no.)	120.2 (3.0)	63.7 (3.6)	0.02
Sleep stage transition (no.)	335.9 (2.8)	169.7 (4.6)	0.01
Stage 1 (%)	18.2 (0.5)	12.4 (1.2)	n.s.
Stage 2 (%)	54.2 (0.9)	44.9 (1.8)	n.s.
Stages 3 and 4 (%)	11.9 (1.0)	13.7 (1.5)	n.s.
REM (%)	19.0 (0.6)	29.0 (1.7)	0.04
PLMW index (n/h)	28.5 (1.4)	4.5 (1.14)	0.01
PLMS with MA index stage 1 (n/h)	47.5 (1.6)	49.9 (2.4)	n.s.
PLMS without MA index stage 1 (n/h)	1.2 (0.4)	2.0 (1.1)	n.s.
PLMS with MA index stage 2 (n/h)	38.4 (1.3)	36.8 (2.6)	n.s.
PLMS without MA index stage 2 (n/h)	26.2 (1.2)	16.5 (1.6)	0.04
PLMS with MA index stages 3 and 4 (n/h)	1.7 (0.6)	1.1 (0.4)	n.s.
PLMS without MA index stages 3 and 4 (n/h)	46.8 (2.4)	41.8 (3.6)	n.s.
PLMS with MA index stage REM (n/h)	29.8 (1.6)	12.4 (2.3)	0.04
PLMS without MA index stage REM (n/h)	11.5 (1.2)	5.6 (1.5)	n.s.

PLMW, periodic leg movements during wakefulness; PLMS, periodic leg movements during sleep; PLMS with MA, periodic leg movements during sleep with microarousal; PLMS without MA, periodic leg movements during sleep without microarousal. *P*, Mann–Whitney U test.

Table 2

Number and duration (mean ± SEM) of considered periodic leg movements and associated microarousals during stages 1 and 2 of NREM sleep, REM sleep and wakefulness

		Wake	Stage 1	Stage 2	REM
PLM with MA	Number	211	677	1666	399
	Duration (s)	2.95 ± 0.24	2.96 ± 0.21	3.17 ± 0.22	2.44 ± 0.23
	Index (n/h)	2.45 ± 0.28	8.14 ± 1.54	18.87 ± 2.00	4.91 ± 1.25
MA	Duration (s)	–	6.05 ± 0.14	5.31 ± 0.15	4.80 ± 0.16
PLM without MA	Number	–	–	740	–
	Duration (s)	–	–	2.72 ± 0.16	–
	Index (n/h)	–	–	9.88 ± 1.37	–

PLM, periodic leg movement; MA, microarousal.

patients showing a significant rise in the PLMW index and in the PLMS index during REM sleep and stage 2.

Three thousand six hundred and ninety-three PLM were analyzed (Table 2) representing 79.5% of the total number

of PLM. No significant difference in PLM duration were found between stages, despite PLM tended to be shorter in REM sleep stage ( $P = 0.07$ ). No difference in duration was found for PLM with and without MA in stage 2. One-way

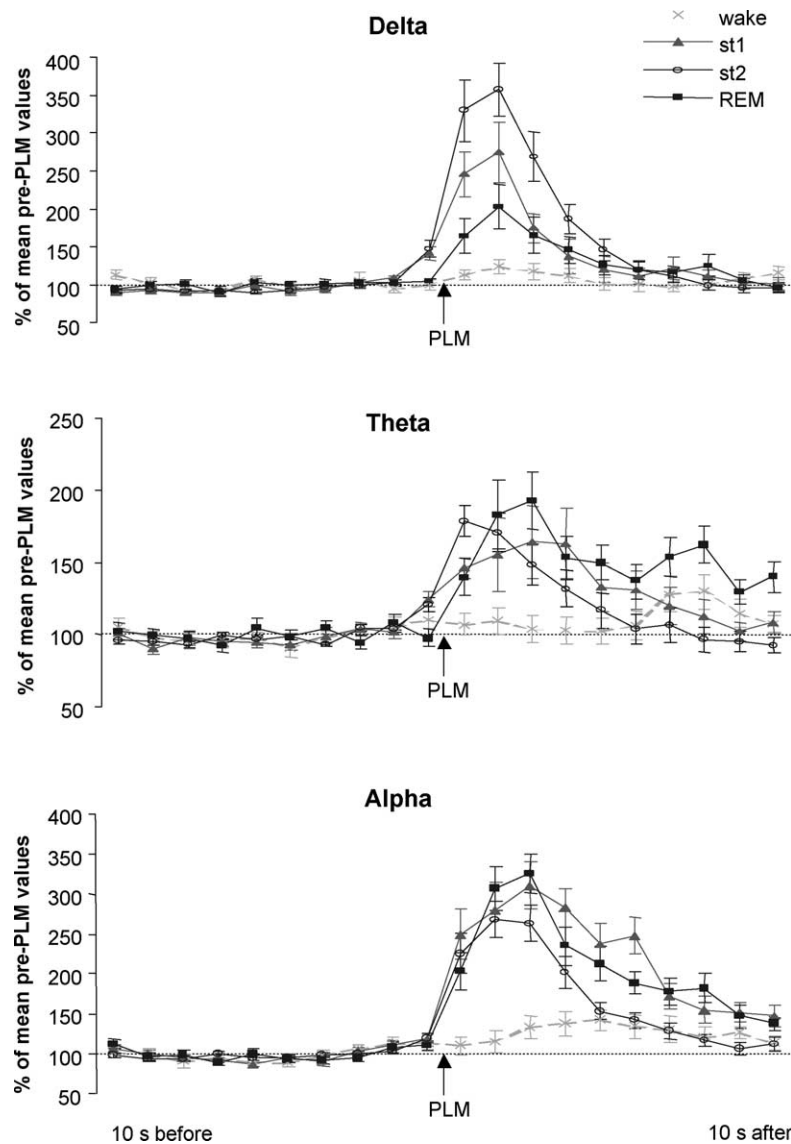


Fig. 1. Time course of EEG spectral power in delta, theta and alpha frequency bands 10 s prior and 10 s after the PLM onset (black arrow) during wakefulness, stages 1, 2 and REM sleep stage for PLM with MA. Values are expressed as a percentage of the mean value during pre-PLM period.

ANOVA for repeated measures revealed a stage effect for MA duration ( $F(2, 22) = 59.4, P < 0.0001$ ), MA tending to be longer in stage 1 and progressively shortening in stage 2 ( $P = 0.0001$ ) and in REM sleep ( $P = 0.0007$ ).

3.2. Spectral EEG analysis

The mean profiles of EEG spectra in delta, theta, alpha, sigma, beta1 and beta2 bands for 10 s before and 10 s after PLMW and PLMS with MA onset during wakefulness and each considered sleep stages are shown in Figs. 1 and 2. The data indicate the presence of a transient EEG activation, during the first 4 or 5 s after the PLM onset, that was dependent on stage effect, wakefulness having minor or little effect on EEG powers.

During wakefulness, a small and not significant increase in cerebral activity was present, starting at the 1st-s window

after the PLMW onset and persisting in the post-PLMW period. In contrast, a clear time effect in all frequency bands was observed during PLMS with MA occurring in stages 1 ( $P < 0.0001$ ), 2 ( $P < 0.0001$ ) and REM sleep ( $P < 0.0001$ ). The magnitude and the pattern of the slow and fast EEG responses was large and sustained in all sleep stages with differences, however, between stages as well as between the different frequency bands. As shown by Fig. 1, in stages 1 and 2, the pattern of EEG activity was remarkably stable up to the 1st-s window before the PLMS onset, at which point a clear EEG change in slow wave activities occurred, characterized by an increase in delta (stage 1:  $P = 0.02$ ; stage 2:  $P = 0.004$ ) and in theta (stage 1:  $P = 0.008$ ; stage 2:  $P = 0.03$ ) bands compared to REM sleep. During stage 1, this increase remained significantly higher compared to pre-PLMS values for the windows 1–3 in the delta band ( $P < 0.05$ ) and for

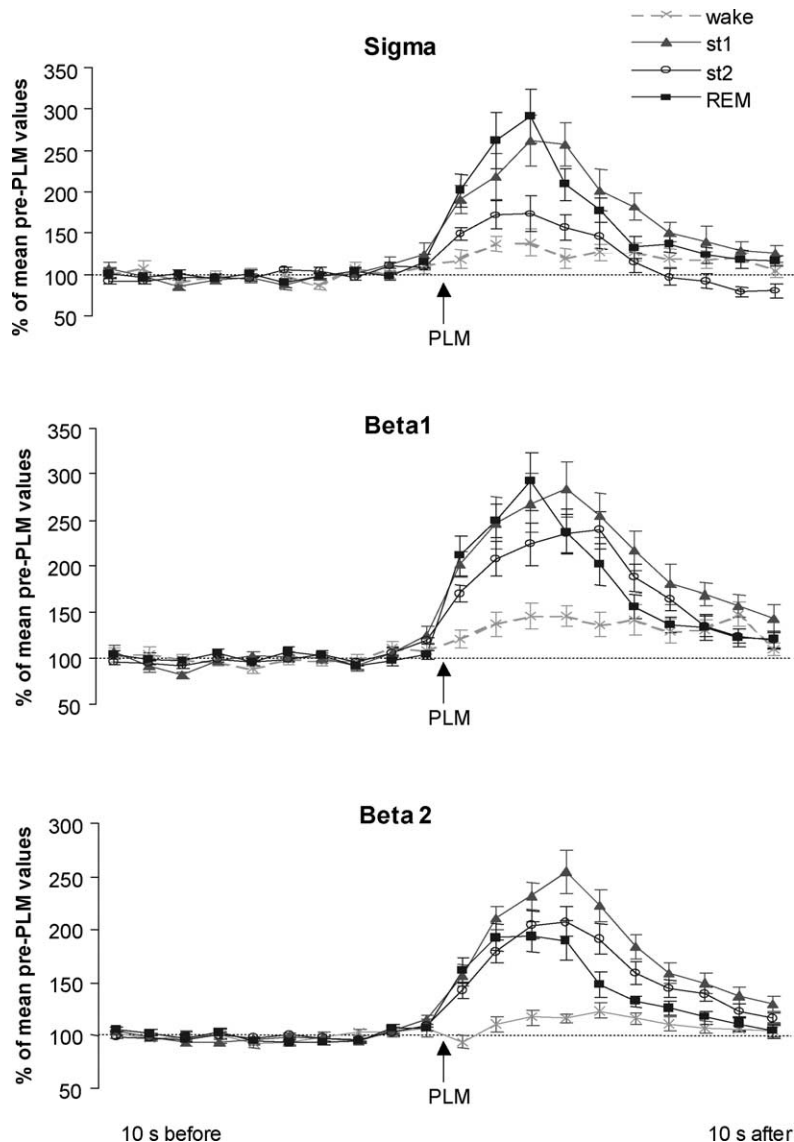


Fig. 2. Time course of EEG spectral power in sigma, beta1 and beta2 frequency bands 10 s prior and 10 s after the PLM onset (black arrow) during wakefulness, stages 1, 2 and REM sleep for PLM with MA. Values are expressed as a percentage of the mean value during pre-PLM period.

the windows 2–4 in the theta band ( $P < 0.05$ ). In stage 2, the delta and theta activity rise was stronger, the first 4-s windows after the PLMS onset being significantly higher than pre-PLMS values (delta:  $P < 0.01$ ; theta:  $P < 0.05$ ) and significantly different from those obtained during REM sleep (delta, windows 1–3:  $P < 0.05$ ; theta, window 1:  $P < 0.05$ ). After the 3rd–4th-s windows, a rapid decline in slow EEG activities was present with a return to baseline values during both stages 1 and 2.

When we consider fast frequencies and NREM sleep, we note that the activity in all fast frequencies significantly increased during the first 3-s windows after the PLMS onset with a tendency to sustained increase in stage 1 and a return to baseline values in stage 2. Alpha activity (Fig. 1) strongly increased during the first 4-s windows compared to baseline values (stage 1:  $P < 0.0001$ ; stage 2:  $P < 0.0001$ ) with

a persistent activation in stage 1 during all the post-PLMS period significant until the 6th-s window ( $P < 0.0001$ ). Sigma, beta1 and beta2 activities (Fig. 2) followed about the same pattern than alpha activity with an increase during the first 4-s windows compared to pre-PLMS values ( $P < 0.05$ ), this increase being greater in stage 1 compared to stage 2 for the sigma band ( $P < 0.05$ ). The initial increase was then followed by a decrease toward baseline values in stage 2, but with a trend of maintained increase in stage 1.

During REM sleep (Figs. 1 and 2), the EEG changes followed about the same pattern than that found in stages 1 and 2, but with differences in the pre-, during- and post-PLMS period. In contrast to NREM sleep where the activity in the slow frequencies began to increase in the window 1-s before the onset of the PLMS, during REM sleep, the activity in all frequency bands started to increase at the 1st-s

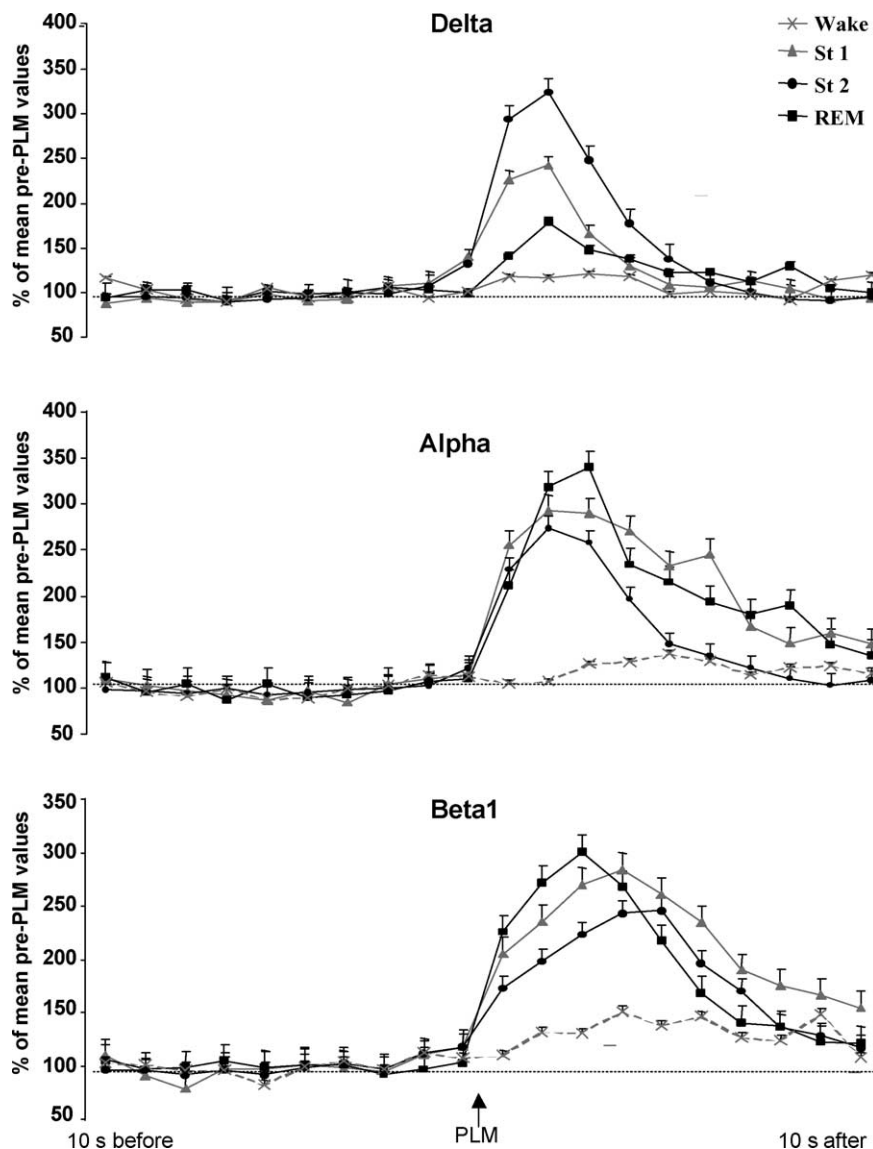


Fig. 3. Time course of EEG spectral power in delta, alpha and beta1 frequency bands 10 s prior and 10 s after the PLM onset (black arrow) during wakefulness, stages 1, 2 and REM sleep for PLM with MA in patients with restless legs syndrome.

window after the PLMS onset. The rise reached a peak between the 2nd-s and the 3rd-s window after PLMS onset and progressively declined to baseline values. However, theta, alpha and sigma activities showed sustained elevation during all the post-PLMS period with values being significantly more elevated than baseline values until the 8th-s window ( $P < 0.05$ ). This trend was not observed in the delta and beta bands.

To take into consideration the possible effect of diagnosis on the described differences between wakefulness and sleep, the same ANOVA design was performed considering only PLMW and PLMS with MA in patients with RLS. As shown in Fig. 3, the results of this ANOVA completely paralleled those previously described. In RLS patients, ANOVA revealed a similar pattern of EEG variations with a significant time effect (delta:  $F = 27.8$ ,  $P < 0.0001$ ; theta:

$F = 10.2$ ,  $P < 0.0001$ ; alpha:  $F = 48.5$ ,  $P < 0.0001$ ; sigma:  $F = 41.4$ ,  $P < 0.0001$ ; beta1:  $F = 54.9$ ,  $P < 0.0001$ ; beta 2:  $F = 82.0$ ,  $P < 0.0001$ ) and a significant sleep stage interaction (delta:  $F = 5.1$ ,  $P < 0.0001$ ; theta:  $F = 2.3$ ,  $P < 0.0001$ ; alpha:  $F = 5.4$ ,  $P < 0.0001$ ; sigma:  $F = 5.1$ ,  $P < 0.0001$ ; beta1:  $F = 4.3$ ,  $P < 0.0001$ ; beta 2:  $F = 9.2$ ,  $P < 0.0001$ ), again wakefulness having minor or little effect on EEG power changes.

Fig. 4 shows the changes in delta, alpha and beta1 power for PLMS without MA occurring in stage 2 compared to PLMW. Again a typical time effect was noted only for PLMS without MA ( $P < 0.0001$ ), wakefulness inducing little effect on EEG powers. Appearance of PLMS without MA induces a rise in all considered powers at the PLM onset with a greater increase during the first 3 or 4 s and followed by a return to baseline values.

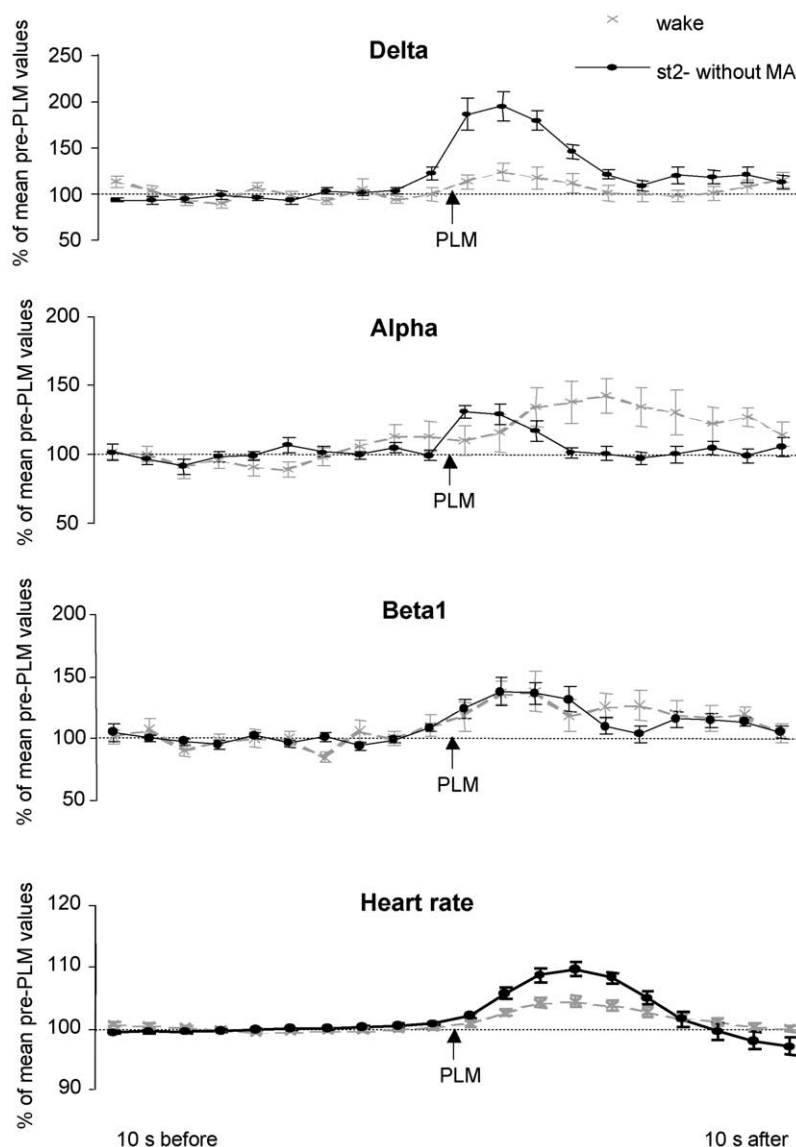


Fig. 4. Over-time changes in delta, alpha and beta1 frequency bands and heart rate in the pre- and post-PLM period during wakefulness and sleep stage 2 for PLM without MA. The black arrow indicates the motor event onset.

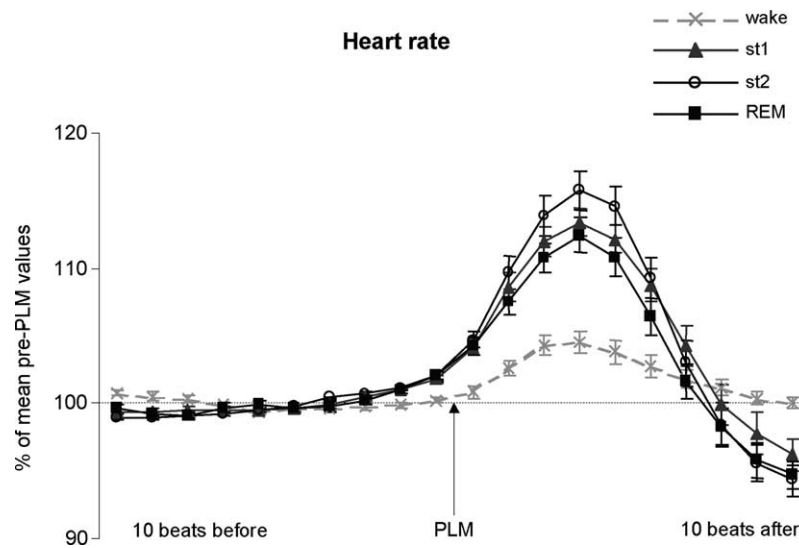


Fig. 5. Heart rate variations 10 beats prior and 10 beats after the PLM onset (black arrow) during wakefulness, stages 1, 2 and REM sleep stage for PLM with MA and PLMW.

These changes, however, were significantly lower ( $P < 0.05$ ) compared to PLMS with MA. When changes during wakefulness were compared to those occurring around the PLMS without MA, the magnitude of power variations were greater during sleep than awake state, two-way ANOVA revealing, however, statistical significant differences only for the delta band ( $P < 0.0001$ ).

### 3.3. Heart rate analysis

HR variations around PLM with MA occurring during the 3 sleep stages as well as during wakefulness for the 20 windows analysis period are shown in Fig. 5. A two-way ANOVA revealed a significant effect for the factors time ( $P = 0.005$ ) and stage ( $P < 0.00001$ ), indicating that the pattern of the autonomic response was dependent on the occurrence of the motor phenomena but its magnitude depended on its occurrence during sleep or wakefulness. During wakefulness, a tachycardia was observed, starting with the onset of the PLMW, and being significantly different than pre-PLMW values from the second to the seventh beats ( $P < 0.05$ ). During sleep, the HR response profile was similar over the 3 sleep stages, characterized by a rise two beats before the onset of the PLMS and the MA, peaking at the fourth beat after the PLM onset, and followed by a decrease to values below the baseline values. The increase was significant from the beats 1 to 6 post-PLMS for stages 2 ( $P < 0.01$ ) and REM ( $P < 0.05$ ) and until the seventh beat for stage 1 ( $P < 0.05$ ). Subsequently, the HR values decreased to below baseline values and became significantly ( $P < 0.05$ ) lower compared to pre-PLMS values at the ninth beat for stages 2 ( $P < 0.01$ ) and REM sleep ( $P < 0.05$ ) and at the 10th beat for stage 1 ( $P < 0.05$ ). Inspection of Fig. 3 reveals that the pattern of HR changes was similar for PLMS without MA showing a rise in the HR response from the 2nd to the 6th beats ( $P < 0.01$ ), with

a return to baseline levels, again statistically different compared to wakefulness ( $P < 0.0001$ ) and showing a trend to be lower compared to PLM with MA ( $P < 0.06$ ).

## 4. Discussion

Our study is the first to investigate the autonomic and cerebral responses to PLMS and wakefulness in order to assess whether the occurrence of PLM is associated with a pattern of EEG and ECG variation similar in all considered sleep stages and independent of its occurrence during sleep or wakefulness. The first finding of the current study is that the time course of EEG response was substantially different between wakefulness and sleep, independent of the occurrence of MA, with differences related to sleep stages. While in stages 1 and 2 of NREM sleep, the PLM was preceded by a rise in slow EEG activity paralleling the rise in HR, in wakefulness and REM sleep, the changes in cerebral activity appeared simultaneously with the PLM onset. Second, while during sleep, the EEG variations were strong and persistent, during wakefulness the EEG activation was almost absent. Third, despite the small sample of PLMD patients examined, the differences between wakefulness and sleep appear to be similar in RLS and PLMD suggesting an underlying common pathogenetic mechanism. Finally, while a typical pattern of cardiac response was present during NREM and REM sleep, the autonomic activation was smaller but prolonged during wakefulness. These results lead us to think that the mechanisms triggering PLM are different between NREM sleep, REM sleep and wakefulness, suggesting that the occurrence of PLM may be underlie by specific sleep state-dependent mechanisms.

Previous investigations on PLM have predominantly been performed only in light sleep (Pollmächer and Schulz, 1993; Sforza et al., 1999) with few studies

(Montplaisir et al., 1985; Nicolas et al., 1999) considering both sleep and wakefulness. Nicolas et al. (1999) found sleep–wake states-related differences in PLM duration and periodicity, PLM being longer in waking state and showing a smaller frequency during REM sleep. However, analysing the periodicity of PLMS and wakefulness, the authors proposed that all PLM share pathophysiological similarities, possibly involving a common generator located at spinal level (Dickel et al., 1994; Yokota et al., 1991). This generator would be active during both wakefulness and sleep, modulated by descending dopaminergic pathways (Quatralo et al., 2003; Trenkwalder et al., 1999), and paralleling the periodicity of other systems. In contrast, our results showed two different patterns of cerebral and autonomic response during sleep and wakefulness. During sleep, we noted a bimodal response in EEG spectral power and HR variability, consisting of a rise in cerebral and autonomic activity around the PLM, peaking between the windows 2 and 4 after the PLM onset, and followed by a progressive return to baseline values for EEG activity and a fall below baseline values for HR. On the other hand, during wakefulness the pattern of HR and EEG responses was characterised by a much smaller rise and no decrease of the HR variability below baseline values. Therefore, different mechanisms may modulate the cerebral and cardiac response to PLM depending on its occurrence during wakefulness or during sleep. A recent study on cardiac activity during wakefulness and sleep (Trinder et al., 2003) has demonstrated a different pattern of cardiac response when arousal occurred in association to spontaneous or evoked stimuli. In line with these data, our results support the hypothesis that the ‘arousal state’ is a waking reflex condition, neurophysiologically different from sustained wakefulness (Horner et al., 1995; Trinder et al., 2001, 2003). During wakefulness, as a consequence of alertness state, cortical and cardiac activity are set at a waking level that does not allow further increase in EEG activity and HR at the occurrence of the motor stimuli. In contrast, during sleep the PLM and the associated MA would induce a transitory ‘arousal state’ inducing an activation and thereafter a return to basal levels as a consequence of the return to sleep.

The different pattern of EEG changes occurring around PLMW, opens discussion on the pathophysiologic mechanisms underlying the appearance of PLMW in RLS patients. A limb movement, either voluntary (Neuper and Pfurtscheller, 1996) or passive (Cassim et al., 2001) is accompanied by changes in cortical activity consisting of a decrease in alpha and beta activity 1 s before the movement onset followed by an increase in these bands in the post-movement period. If the pre-movement changes have been related to motor preparation, the post-movement powers increase could be related to the return of the motor cortex to rest (Pfurtscheller et al., 1996) or closure of the motor process (Alegre et al., 2002) or to sensory afferences (Cassim et al., 2001). In our patients, we found a small and not significant rise in all frequency bands starting after

the PLMW onset and lasting until the end of muscle contraction, thus similar to that described during a voluntary or passive limb movement. Despite the methodological limitation of our analysis, not including event-related synchronization, our results may support the hypothesis of an increased motor excitability inducing a higher need for cortical inhibition (Schober et al., 2004) in RLS patients during both voluntary (Schober et al., 2004) and involuntary movements (Rau et al., 2004).

Another interesting finding of our study is that significant differences between light NREM sleep and REM sleep occur before and during the PLM, independently of the arousal and PLM duration. The present findings replicate those obtained during a study conducted in NREM sleep by our group (Sforza et al., 2002). In fact, while in stages 1 and 2, the activity in slow EEG bands started to rise 1 s before the PLM onset, peaked at the 2nd to 4th-s window after the onset, and thereafter returned to baseline values, in REM sleep the rise occurred simultaneously with the motor event onset and remained at higher levels across and after the PLM, even though shorter was the arousal and the PLM durations. These results also have implications for elucidating the mechanisms underlying occurrence of PLM and associated arousal from sleep. There is convergent evidence (Karadeniz et al., 2000; Montplaisir et al., 1994b; Warnes et al., 1991) that PLMW and sleep may be considered more as a sign of sleep instability than a motor disorder. The MA occurring along with the PLMS is often detected before the onset of the motor event during which K-complexes may appear (Karadeniz et al., 2000). Moreover, K-alpha activity may appear prior to the PLMS onset with a rate and a periodicity that remains unchanged after reduction of PLMS rate with L-dopa (Montplaisir et al., 1996). Finally, periodic spontaneous MA is observed in patients showing PLMS associated with MA during the same night (El-Ad and Chervin, 2000) or the night before (Haba-Rubio et al., 2002). Thus, a central nervous oscillating system may be present during sleep, triggering the MA, the PLMS or both (Karadeniz et al., 2000) and translated at the EEG level by the cyclic alternating pattern (CAP; Parrino et al., 1996; Terzano and Parrino, 1993; Terzano et al., 1985) and at the autonomic level by a rise in HR (Ferri et al., 2000). Ninety-six percentage of PLMS occur during the phase A of the CAP (Parrino et al., 1996), in a manner similar to that described for other phasic sleep events such as apneas (Terzano et al., 1996) or seizures (Parrino et al., 2000). The presence of a transitional phase between wake and sleep facilitating the onset of PLM may also be proposed during wakefulness (Montplaisir et al., 1994b), periodic oscillatory changes in the EEG activity, as assessed by the slow fluctuations of the ratios alpha + beta/delta + theta, being present in association with the PLMW.

If the hypothesis of oscillatory processes occurring during wakefulness and sleep and generating both the PLM and the MA is correct, we would expect a common pattern of changes in spectral EEG activities occurring

before the PLM and affecting all frequency bands. In contrast to this hypothesis, we observed a pre-PLM rise only in slow wave activities and this, only during NREM sleep. Despite our results, do not allow us to confirm or reject the sleep instability hypothesis, the sleep state differences in the EEG response to PLM suggest that the mechanisms triggering the PLM during REM sleep and wakefulness might be different from those proposed to be involved in NREM sleep, the only sleep state during which the CAP could be observed (Terzano et al., 1985).

The interpretation of our results may be affected by some limitations of our study design. First, we consider only PLMS during stages 1 and 2 and REM sleep, and we did not analyse the EEG and HR changes occurring during SWS. The fact is that, in line with previous observations (Pollmächer and Schulz, 1993; Sforza et al., 1999), we found a limited number of PLM with MA in SWS, limiting the sample of movements that could be analyzed. Second, our patients sample was smaller and probably not representative of larger RLS and PLMD populations but, the first aim of our study was to obtain a homogenous group having PLM in all considered sleep stages and wakefulness, independently of the effect of other factors such as onset of the disease, age and gender (Gosselin et al., 2003; Nicolas et al., 1999). Third, choosing an EMG definition for PLM onset necessarily relies on arbitrary criteria. We chose to evaluate the PLM onset as a single point time where an increase in EMG was present, this criteria being easily respected from an event, and even from a recording, to another. Finally, since in our group only few patients had PLMD, further studies including larger sample are needed to assess whether the described differences between wakefulness and sleep are specific to RLS or could be also present in PLMD patients.

In conclusion, we observed significant differences in the time course of cerebral and cardiac activation during PLM in relation to different sleep states and wakefulness. Wakefulness appears to induce smaller changes in both systems indicating a mechanism different from that implicated during sleep. The differences across sleep stages in the EEG and cardiac variations suggest that more a sleep stage dependence of arousal response to motor event rather than a common generator may be implicated in the occurrence of PLMS. Further studies using more powerful EEG measures such as event-related potentials and acting on the instability of the common generator will be required to better understand the mechanisms implicated in the genesis of PLM during wakefulness and sleep.

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