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Periodic limb movements both in non-REM and REM sleep: Relationships between cerebral and autonomic activities

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ABSTRACT

Objective: To investigate the temporal relationship between cerebral and autonomic activities before and during periodic limb movements in NREM and REM sleep (PLMS).
Methods: Patterns of EEG, cardiac and muscle activities associated with PLMS were drawn from polysomnographic recordings of 14 outpatients selected for the presence of PLMS both in NREM and REM sleep. PLMS were scored during all sleep stages from tibial EMG. Data from a bipolar EEG channel were analyzed by wavelet transform. Heart rate (HR) was evaluated from the electrocardiogram. EEG, HR and EMG activations were detected as transient increase of signal parameters and examined by analysis of variance and correlation analysis independently in NREM and REM sleep. Homologous parameters in REM and NREM sleep were compared by paired *t*-test.
Results: The autonomic component, expressed by HR increase, took place before the motor phenomenon both in REM and NREM sleep, but it was significantly earlier during NREM. In NREM sleep, PLM onset was heralded by a significant activation of delta-EEG, followed by a progressive increase of all the other bands. No significant activations of delta EEG were found in REM sleep. HR and EEG activations positively correlated with high frequency EEG activations and negatively (in NREM) with slow frequency ones.
Conclusions: Our findings suggested a heralding role for delta band only in NREM sleep and for HR during both NREM and REM sleep. Differences in EEG and HR activation between REM and NREM sleep and correlative data suggested a different modulation of the global arousal response.
Significance: In this study, time–frequency analysis and advanced statistical methods enabled an accurate comparison between brain and autonomic changes associated to PLM in NREM and REM sleep providing indications about interaction between autonomic and slow and fast EEG components of arousal response.

1. Introduction

Periodic limb movements (PLM) are involuntary, non-epileptic, repetitive, stereotyped limb movements that occur mostly during non-rapid eye movement (NREM) sleep (Coleman et al., 1980) and less frequently during rapid eye movement (REM) sleep (Pollmacher and Schulz, 1993; American Academy of Sleep Medicine, 2005). These movements are best described as rhythmic extensions of the big toe and dorsiflexions of the ankle with occasional flexions of the knee and hip.

PLMS were first polygraphically documented in restless legs syndrome – RLS (Coleman et al., 1980; Lugaresi et al., 1986). However, they also occur in a wide range of sleep disorders, including obstructive sleep apnoea syndrome (OSAS) (Fry et al., 1989), narcolepsy (Montplaisir et al., 2000), REM behaviour disorder (Fantini et al., 2002), insomnia (Montplaisir et al., 2000), hypersomnia (Coleman et al., 1980; Montplaisir et al., 2000). The presence of PLM in patients with sleep disorders has been estimated around 25% (Karatas, 2007) but their presence has also been reported in subjects without any other complain of disturbed sleep and their prevalence in the general adult population has been estimated to be 4–11% (Hornyak et al., 2006). This prevalence is correlated with age, in fact it is low in young people while is high in elderly: it has been estimated around 29% for subjects over 50 years and nearly 44% in subject over 65 years. It is low in children, where PLMS have been seen in patients with attention-deficit/hyperactivity disorder and low serum iron/ferritin levels (Pennestri et al., 2006; Karatas, 2007).

The pathogenesis of PLMS is still unclear and dysfunctions at several different levels of neuroaxis have been suggested, including cortical and sub-cortical brain regions, the spinal cord and peripheral nerve (Yokota et al., 1991; Bucher et al., 1997; Tergau et al.,

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1999). A primary involvement of the dopaminergic system has been widely documented with particular reference to RLS (Montplaisir et al., 1991; Staedt et al., 1995; Manconi et al., 2007; Vetrugno et al., 2007).

The relationship between PLMS and daytime sleepiness is controversial and there are studies suggesting that PLMS may not be considered a primary cause of sleep disturbance (Coleman et al., 1982; Nicolas et al., 1998; Chervin, 2001; Karadeniz et al., 2000). However, PLMS are often associated with awakenings or EEG arousals, which cause sleep fragmentation and may lead to non-restorative sleep. With a stress on their periodicity PLMS have been related to the cyclic alternating pattern (CAP), a sequence of biphasic cycles reflecting a condition of unstable sleep in which phasic EEG patterns, such as bursts of delta waves or arousals (phase A), periodically intrude between intervals of background EEG activity (phase B) (Terzano et al., 2002). Parrino et al. showed that PLMS mostly appear during CAP sequences with the great majority of limb movements associated with phase A (Parrino et al., 1996).

The cerebral and autonomic changes associated with PLMS have been studied evaluating heart rate (HR) and EEG spectral power as computed by Fourier analysis (Sforza et al., 2002; Lavoie et al., 2004) and recently using time–frequency EEG analysis by wavelet transform (Ferrillo et al., 2004). These studies showed that PLMS were mostly preceded by autonomic and EEG activations, where EEG activations could be micro-arousals or other phasic patterns, such as burst of slow wave activities, as in the A-phase of a CAP sequence. These patterns have been considered as different levels of arousal responses (Sforza et al., 2002).

In a later study, (Ferri et al., 2007) leg movements (LM) in RLS has been classified as periodic or isolated, monolateral or bilateral and analyzed in both NREM and REM sleep. The early increase of HR and EEG activities, as quantified by Fourier transform, was found for both periodic and isolated movements in NREM, but only for isolated movements in REM, while EEG changes were suggested to precede HR increase. On the contrary, another study applying time–frequency analysis of EEG and spectral analysis of HR variability – and only considering stage 2 NREM sleep – suggested a time sequence starting with cardiac activation followed by an increase of delta EEG and then faster EEG activities (Guggisberg et al., 2007). The fast EEG component (gamma band) is reported to increase before the onset of movement for isolated or breathing-related leg movements, but after the onset of PLMS. In the same study significant correlations were found between cardiac activation and muscle activity as well as between cardiac activation and EEG delta activity but not with faster EEG rhythms.

The aim of this study was to analyze the temporal relationships between autonomic, cerebral and muscle changes associated with PLMS in both REM and NREM sleep. Time–frequency analysis and statistical procedures were applied to detect activations of EEG components, HR and EMG and to provide a quantitative evaluation of their time location and intensity. This enabled the statistical comparison between activations in NREM and REM sleep and among activation characteristics of HR, EMG and EEG components, with the aim to get new findings about the role and interaction of different cerebral and autonomic components involved in the arousal response.

2. Methods

2.1. Subjects

Fourteen outpatients (6 males and 8 females, mean age 56 ± 12.5 years) complaining of bad quality of sleep (mean duration of the disease: 15.2 ± 7.3 years) were enrolled in the Sleep Clinic at the San Martino Hospital in Genoa, during the period between January 1st, 2002 and December 31st, 2006. The presence of PLMS was suggested by anamnestic data and then confirmed by polysomnographic recording. Only patients with PLMS in both NREM and REM sleep were included in the study.

Exclusion criteria were conditions known to be associated with PLM, such as anaemia, diabetes, renal failure, peripheral neuropathy, myelopathy, or the presence of any other neurologic, psychiatric or sleep-related disorders, including breathing disorders. To exclude a PLMW (PLM during wakefulness) each registration was preceded by a SIT (Suggested Test Immobilization). None of the patients had cardiovascular disease or a history of drug overuse. None of the patients was taking any medication (benzodiazepines, carbamazepine, opioids, dopamine agonist or related drugs) which may alter sleep micro or macrostructure or PLM parameters. Written informed consent in accordance with the Declaration of Helsinki 1964 and its 1975 Tokyo amendment was obtained from all subjects. All patients underwent nocturnal polysomnographic recordings – starting at 23:00 and ending at 7:00.

A control group of healthy subjects was drawn from the database of the Centre for Sleep Medicine (DISMR, University of Genoa, Italy) in order to evaluate conventional sleep parameters in the patient group in comparison with reference data. The control group included fourteen subjects in the same age span as patients (6 males and 8 females, mean age 53 ± 11 years) who had undergone polysomnographic recording with the same equipment and recording condition.

2.2. Data recording

Nocturnal sleep was monitored by means of a digital polygraph (Galileo NT, EBNeuro).

Each polysomnogram included electroencephalogram, electrooculogram (EOG)s, submental (EMG) and anterior tibial electromyogram (TB), measurements of oro-nasal airflow (nasal–oral thermistor), chest and abdominal excursion, oxyhemoglobin saturation (finger pulse oxymetry) and single-lead electrocardiogram from a standard (V5) precordial lead (ECG).

EEG was acquired from 4 electrodes (F4, C4, O2, A1), positioned according to the 10–20 International Electrodes Placement System in physical reference with successive reconstruction of bipolar derivations. The low-pass filter was set at 70 Hz, the time constant at 0.1 and the notch filter was switched on. The EMG signal was recorded with a time constant set at 0.01 s and a low-pass filter at 70 Hz. A 50 μV sinusoidal calibration signal of approximately 1 min duration was obtained in all subjects at the start of monitoring. The quality of the EMG recording was ascertained by asking the patient to flex his knees and feet.

All signals were sampled at a 256 Hz frequency by an analog–digital converter with 16 bit resolution. Each record was visually scored according to Rechtschaffen and Kales criteria (Rechtschaffen and Kales, 1968) and then revised following to the new standard (Iber et al., 2007). The hypnograms were stored as digital data within the recording.

According to the new standard (Iber et al., 2007), a leg movement (LM) occurring either in NREM or REM sleep was marked if it lasted 0.5–10 s, being included in a series of at least 4 LM, with a movement interval of 20–90 s. Due to some problems with the application of the absolute threshold proposed in (Iber et al., 2007), as in previous study (Ferrillo et al., 2004), movement onset was detected when the amplitude of EMG activity, as evaluated by an exponential weighted moving average of the rectified signal, increased more than 60% above the preceding background. LM offset was detected when EMG activity steadily lowered below a level corresponding to one half of the mean event amplitude.

The PLMS index (number of PLMS per hour of sleep) was computed for each patient and separately for REM and NREM sleep.

2.3. Data analysis

For each PLMS we analyzed a time window starting 20 s before and ending 30 s after PLMS onset. A PLMS was included in the analysis if it was not preceded or followed by another PLMS by at least 30 s and if the associated time window did not contain neither EEG, ECG or TB artifacts nor changes ascribable to a contiguous PLMS, nor following awakenings.

Data from a bipolar EEG derivation (C4-O2) were analyzed by means of the wavelet transform, a mathematical technique particularly suitable and efficient for the analysis of non-stationary signals in the time–frequency plane (Schiff et al., 1994; De Carli et al., 2004). The discrete wavelet transform was used in this study by means of the recursive application of a pair of half-band mirror filters generating wavelet-coefficient time series for each band in a multi-resolution scheme in which high frequencies are represented with high time resolution and large bandwidth while both time resolution and bandwidth are halved at each step toward lower frequencies (Rioul and Vetterli, 1991). The wavelet bands resulting from such sub-band coding scheme were 64–128, 32–64, 16–32, 8–16, and so on. In order to fit meaningful EEG bands, the wavelet packed approach (Mallat, 1998) was applied to split the 8–16 Hz wavelet band into the alpha (8–12) and sigma (12–16) EEG bands. In order to get a good frequency discrimination the wavelet filters were drawn from the application of the Remez exchange algorithm for orthonormal wavelets proposed by Rioul and Duhamel (1994) for the optimization of frequency selectivity (32 coefficients). From such filter bank each EEG segment was decomposed into six filtered signals for the following frequency bands: 0.5–2 Hz (low delta), 2–4 Hz (high delta), 4–8 Hz (theta); 8–12 Hz (alpha), 12–16 Hz (sigma) and 16–32 Hz (beta). The EEG power distribution was evaluated for each band as function of time as the variance of the filtered signal within a moving window with a size corresponding to the wavelet scale (decreasing with increasing frequency) and a moving step of 0.125 s.

HR was evaluated by measuring the interval between consecutive R-waves of the QRS complexes in the electrocardiogram by an automatic adaptive algorithm (Ferrillo et al., 2004) followed by visual check.

Tibial EMG activity was evaluated as total signal power (variance) within the 0.125-s time window.

The time evolution of cerebral, cardiac and muscle activities associated to each event was so described by the eight time series relevant to the EEG power within the six EEG bands, the heart rate and the total EMG power: a series of 400 values represented the evolution of each of these parameters within the 50-s window surrounding an event with a 0.125 s step.

2.4. Statistical analysis

Descriptive statistic of conventional sleep parameters was performed by evaluating mean values and standard deviation of each parameter and by applying the Student's *t*-test to compare patients with controls.

As for the analysis of EEG and heart rate changes associated to PLMS, for each subject, parameter and condition a mean pattern was evaluated by averaging the time series associated to all selected PLMS. We defined (a) parameters: the EEG activity for each frequency band, HR and muscle activity; (b) conditions: NREM or REM sleep; (c) mean patterns: the averaged temporal evolution of each parameter. In this way we obtained a new time series (sequence of data points) with a mean value for each point within the 50-s time window. The mean patterns relevant to each subject were then normalized by computing, for each parameter, a baseline mean value from the first 10 s of the time window and expressing all values in the whole series as percent of this mean.

The presence of activations, seen as a transient increase of the values of a parameter in association with a PLM, was assessed by mean of a linear mixed model, independently applied to each parameter, assuming the time and the REM/NREM condition as fixed effects and the subject as random effect. In order to account for autocorrelation of the time series, a first order autoregressive process was assumed for time dependency and included in the model.

For parameters and conditions in which the effect of time was significant, the time interval characterized by event-related activation was assessed in the following way: a two level confidence interval was evaluated for each subject, parameter and condition, including values falling within two/three standard deviations (SD) from the mean background value, as evaluated considering the first 10 s of the averaged pattern. An activation was detected when parameter values continuously exceeded 3-SD limit (corresponding to a nominal significance level of 0.01) for more than 1 s, but its boundaries were extended as far as the 2-SD limit was reached. Such extension was adopted in order to take the different time constants characterizing different parameters into consideration (fast EEG activities reach their thresholds more quickly than slow EEG or HR).

Onset time and duration of the activation were then computed along with its energy (evaluated as the area under the curve between the onset and the end of the activation) and barycenter (a mean time in which each time point within the activation contributes with a weight proportional to the parameter value). The distribution of each one of these measures characterizing activation was tested for normality by the Shapiro–Wilk test.

The differences among parameters concerning activation onset time, duration, energy and barycenter were studied independently for REM and NREM sleep by means of analysis of variance (ANOVA). When ANOVA resulted significant, a contrast analysis was applied following Bonferroni correction.

Paired *t*-test was used to study the differences between REM and NREM sleep for each parameter.

The relationships between cerebral, autonomic and motor activities were further examined by computing the Pearson correlation coefficients between the activation energies for each parameter pair.

In order to keep down the risk of false positive results in presence of multiple tests, all differences and correlations were regarded as significant when they reached a $p < 0.01$ level. The data analysis software R was used for statistical analysis (R Development Core Team, 2006).

3. Results

The mean values of conventional sleep parameters are reported in Table 1, as compared with homologous values in the control group, indicating a poor sleep, pointed out by low sleep efficiency, high wake time after sleep onset and increased number of awakenings. A different sleep structure was suggested in particular by the decrease of percent incidence of slow wave sleep and increased light sleep (N1), which, however, did not reach statistical significance probably due to high variability in patient data. PLM indexes were obviously minimal in the control group and important in patients. PLM index in this group of selected subjects was not much lower during REM than during NREM sleep. The overall number of selected PLMS was 554, among which 438 were found in NREM and 116 in REM sleep.

EEG and heart rate changes associated to a leg movement are illustrated by examples in Fig. 1. During NREM sleep (Fig. 1a) a burst of slow waves surround the beginning of tibial-muscle activity increase and is followed by the increase of EEG frequency while

Table 1
Mean values and standard deviations of conventional sleep parameters characterizing polysomnographic recordings in the group of 14 insomniac subjects and in the matched control group of healthy subjects. Between-group comparisons of mean values were performed for each parameter by Student's *t*-test. Descriptive probability levels are reported and marked by '***' if $p < 0.01$.

	Controls		Patients				
	Mean	St. dev.	Mean	St. dev.	*T*	*P*	
TST	431.36	16.28	331.3	75.7	−4.83	<0.0001	***
Persistent sleep latency (min)	12.39	8.99	23.39	31.69	1.24	0.22	
REM latency (min)	73.12	18.62	71.9	41.34	−0.10	0.92	
Sleep efficiency	0.90	0.03	69.02	16.85	15.13	<0.0001	***
N1%	2.43	1.31	5.18	8.84	1.15	0.26	
N2%	49.51	4.73	56.57	12.76	1.94	0.06	
N3%	23.11	3.73	16.83	12.89	−1.75	0.09	
REM%	24.91	3.41	21.35	10.83	−1.17	0.25	
Wake after sleep onset (%)	4.94	2.40	28.3	16.68	5.19	<0.0001	***
No. of awakenings	16.29	9.10	42.63	13.87	5.94	<0.0001	***
PLM index – REM	0.36	0.93	10.95	8.51	4.63	<0.0001	***
PLM index – NREM	1.50	2.10	12.25	7.26	5.32	<0.0001	***

during REM sleep (Fig. 1b) only the increase of EEG frequency can be observed. In both cases an important increase of heart rate surrounds muscle activation.

The analysis of time patterns by the mixed model indicated significant time effect ($p < 0.01$) – suggesting the presence of an activation – associated to PLMS for all parameters (power of EEG bands, HR, and EMG activity) in NREM sleep and for all parameters but delta power in REM sleep. The averaged profiles for each parameter in NREM and REM sleep are shown in Fig. 2a and b, where activation intervals are marked by a black bar. The activation characteristics – onset time, energy, duration and barycenter – where then analyzed for all parameters excluding delta powers

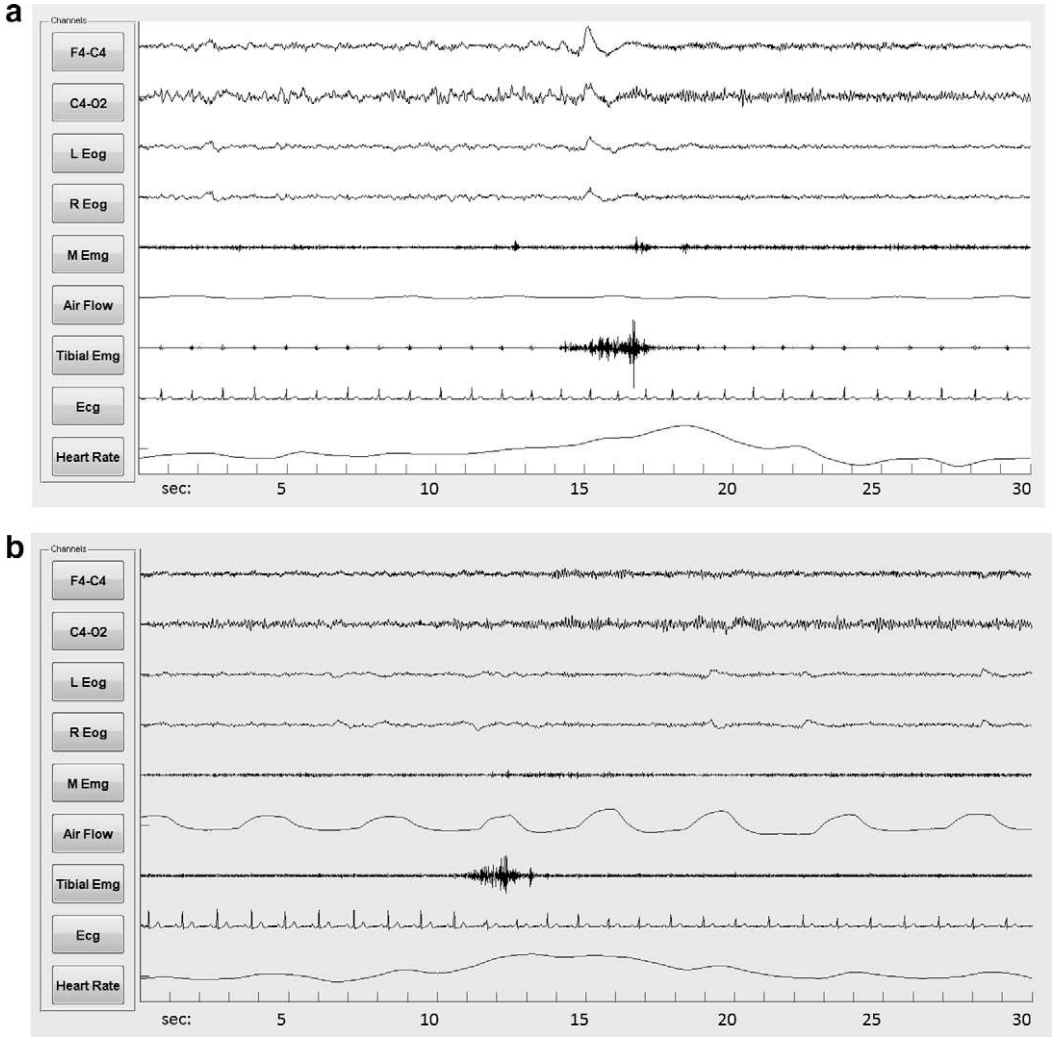

Fig. 1. Example pages of polysomnographic signal associated to a leg movement. The traces are relevant to two electroencephalographic derivations, left and right electrooculogram, submental muscle, air flow, tibial muscle, electrocardiogram and heart rate. (a) NREM sleep. (b) REM sleep.

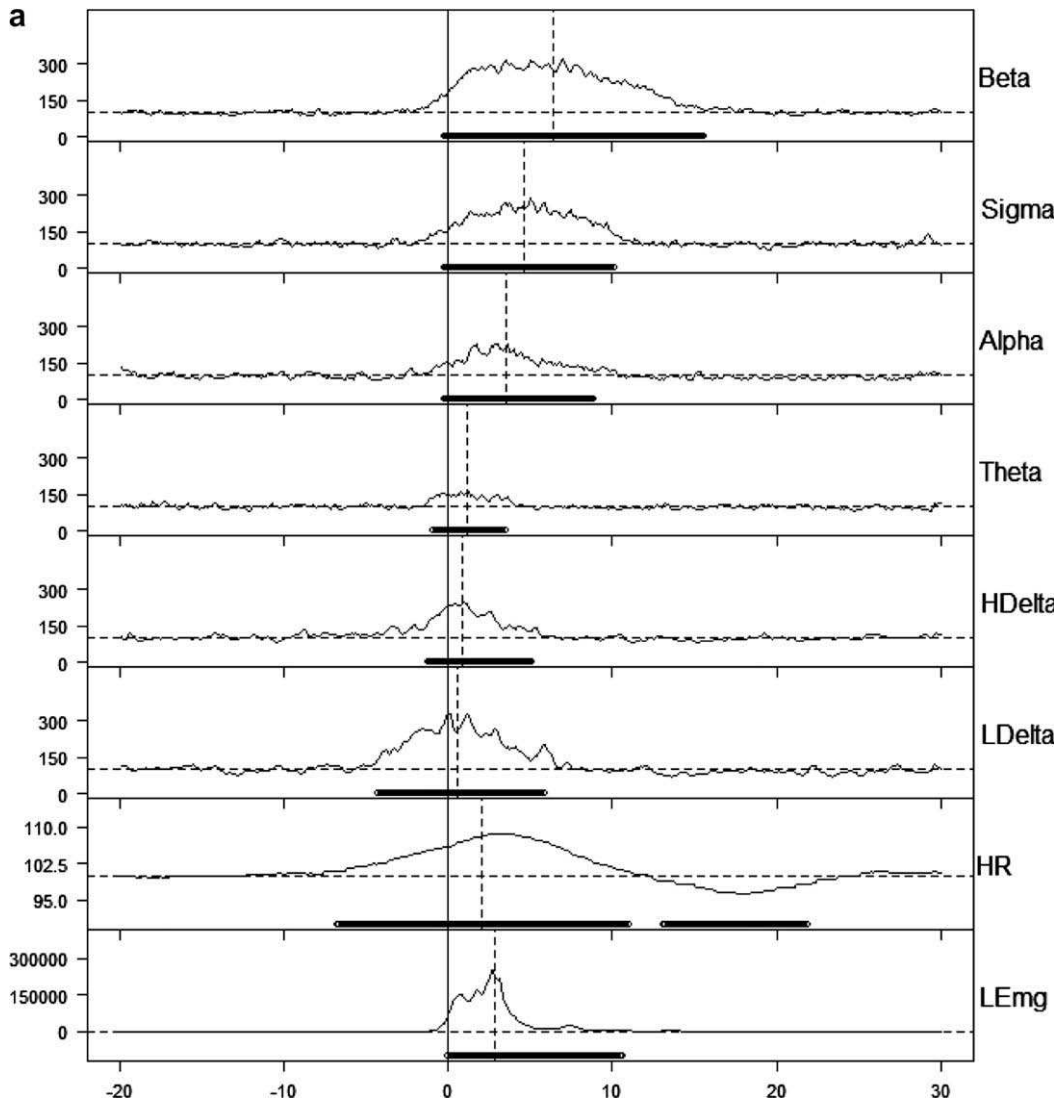

Fig. 2. Mean time course of EEG band power, heart rate and leg EMG power within a time window including 20 s before and 30 s after PLM onset. EEG power has been evaluated by wavelet transform for beta (16–32 Hz), sigma (12–16 Hz), alpha (8–12 Hz), theta (4–8 Hz), high delta (2–4 Hz) and low delta (0.5–2 Hz). Time resolution is 0.125 s and each value is expressed as percent of the mean baseline value, as evaluated considering the time interval from 20 to 10 s before PLM onset. The horizontal black bars indicate significant activations, the vertical dashed bars mark the barycenters. (a) NREM sleep. (b) NREM sleep.

in REM sleep. Their frequency distributions were not significantly different from normal, thus enabling reliable application of the following parametric test. Differences among parameters, as evaluated by ANOVA (Table 2), were significant ($p < 0.01$) for both NREM and REM sleep and for all activation characteristics. The relevant mean values are showed in Fig. 3 along with their (within parameter) standard error.

3.1. NREM sleep

In NREM sleep HR activation significantly preceded all other activations, with a mean onset time of 7.3 s before leg movement onset, followed by low-delta activation whose mean onset time was 3.5 s before movement (Figs. 2a and 3). The activations of other EEG bands showed mean onset times slightly and not significantly preceding movement onset.

The mean duration of HR activation (20.8 s) was significantly longer than durations relevant to EEG and EMG activations, but for HR a shorter period of significant decrease (bradycardia) followed the activation. Among EMG and EEG band activations, a longer duration was observed for fast EEG (alpha, sigma and beta) with respect to delta, but the only significant difference was between beta and high-delta activations.

The barycenters showed a progressive shift of EEG activations with later times for increasing frequencies, from 0.6 s for low-delta to 6.4 s for beta band, while the barycenter of HR activation was at 2.1 s.

As for activation energies, significant positive correlations were found between leg EMG and HR ($r = 0.88$), leg EMG and EEG beta band ($r = 0.89$) as well as between HR and EEG beta band. The activation of EEG beta band positively correlated with the other fast EEG activities: sigma ($r = 0.96$) and alpha ($r = 0.87$), but negatively correlated with activation in high ($r = -0.92$) and low ($r = -0.94$) delta bands. Also leg EMG and HR activation energies negatively correlated with high ($r = -0.82$ and $r = -0.88$, respectively) and low ($r = -0.86$, $r = -0.89$, respectively) delta EEG.

3.2. REM sleep

In REM sleep HR activation significantly preceded all other activations, with a mean onset time of 2.0 s before movement onset

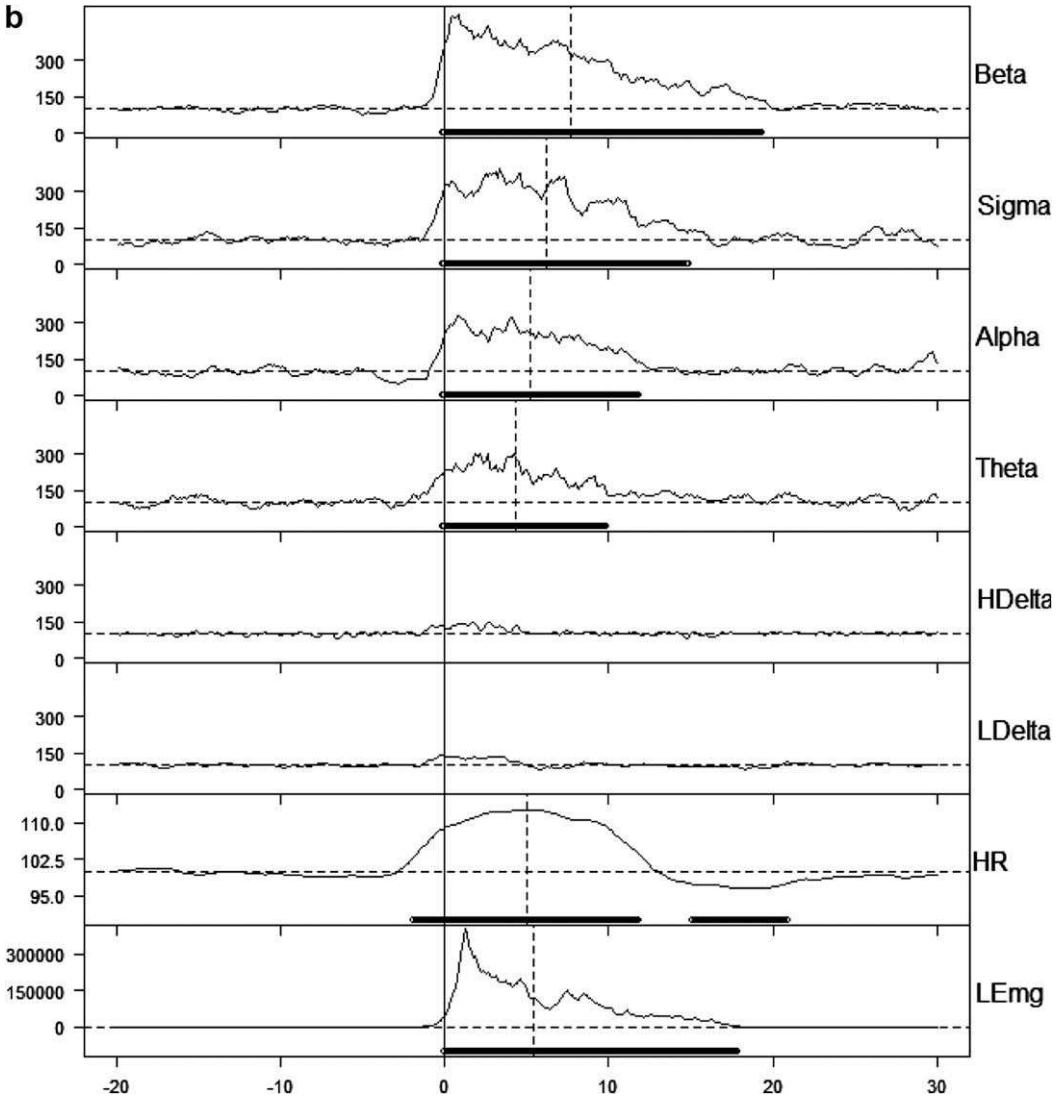

Fig. 2 (*continued*)

(Figs. 2b and 3). The activations of fast EEG bands showed mean onset times slightly and not significantly preceding movement onset while delta bands exhibited only a slight increase of mean activity before movement which did not establish a significant activation.

The mean duration of EEG activation increased with the frequency of the band becoming longer than leg EMG and HR activation for the beta band (20.5 s). In a similar way the barycenters of (fast) EEG activation increased with the band frequency, from 4.3 s for theta to 7.7 s for beta band while mean barycenter for HR was 5.0 s. Also during REM sleep HR activation was followed by a shorter but significant HR decrease (bradycardia).

As for activation energies, significant positive correlations were found between leg EMG and HR ($r = 0.90$), leg EMG and EEG beta band ($r = 0.88$) as well as between HR and fast EEG bands ($r = 0.93$ for beta, $r = 0.88$ for sigma and $r = 0.97$ for alpha). The activation of EEG beta band positively correlated with the other fast EEG activities: sigma ($r = 0.91$) and alpha ($r = 0.90$), while no activation was present for delta bands.

Table 2
Results of analysis of variance (ANOVA): they were highly significant for all activation characteristics (onset, duration, energy and barycentre) indicating differences among mean values relevant to different parameters (EEG band power, heart rate and leg-muscle activity).

	Measures characterizing activation	*F*	*P*
NREM	Onset	42.02	$P < 0.01$
	Duration	14.81	$P < 0.01$
	Energy	41.59	$P < 0.01$
	Barycenter	25.33	$P < 0.01$
REM	Onset	104.24	$P < 0.01$
	Duration	123.70	$P < 0.01$
	Energy	53.63	$P < 0.01$
	Barycenter	40.96	$P < 0.01$

3.3. Comparison between NREM and REM sleep

As for the direct comparison between NREM and REM sleep (Table 3), the first finding was the presence of significant delta EEG activation in NREM but not in REM. The higher frequency EEG bands exhibited stronger activations in REM sleep, expressed by significant increase of both duration and energy and also explaining barycentric time increase while onset time did not show any significant differences. HR activation was earlier (4.89 s mean difference) and its duration longer (2.58 s) in NREM but its energy was higher (+20%) in REM sleep. Mean values of TB activation en-

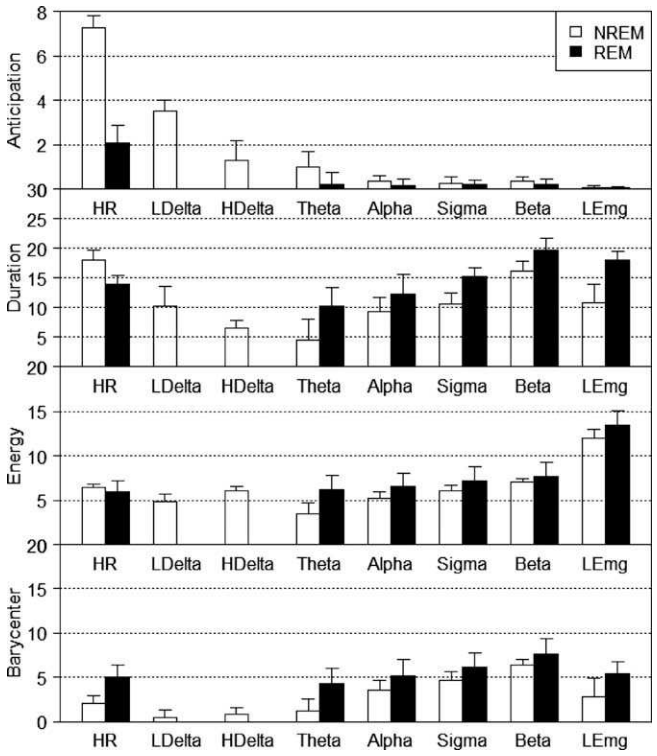

Fig. 3. Mean activation characteristics for all parameters (heart rate, EEG band power and tibial muscle power) during NREM (empty bars) and REM (black bars). Activation is seen as transient increase of the parameter and is defined as described in the text. The between subject standard error is showed for each parameter and condition (NREM and REM).

ergy and duration were also significantly higher in REM than in NREM sleep.

4. Discussion

The association between PLMS and transient increase of autonomic and cerebral activities, which had been previously observed (Sforza et al., 2002; Lavoie et al., 2004; Ferrillo et al., 2004; Ferri et al., 2007; Guggisberg et al., 2007) were further confirmed in this study, which highlighted the following characteristics:

- HR increase, expressing changes in autonomic activity, was found to start some seconds before PLM and also before EEG activation in NREM and, to a lesser extension, in REM sleep.
- In NREM sleep a significant increase of slow EEG activity (reflected by the transient increase of delta, particularly low-delta power) was found before PLM and slightly after HR increase, but no significant activation of delta EEG was found in REM sleep.
- Fast EEG activities (reflected by alpha, sigma and beta power) increased in both NREM and REM sleep, without significant time displacement with respect to PLM; mean energy of the activation was higher in REM than in NREM.
- The strength of leg EMG and HR activations increased together with fast EEG activities, but decreased with slow-EEG activation (delta band).

The increase of HR and delta band before PLMS has already been observed in the above mentioned studies, supporting the hypothesis that PLMS are not the trigger phenomenon for cerebral and autonomic activation: they instead seem ruled by a central oscillatory mechanism regulating both EEG and autonomic functions which can periodically generate conditions facilitating PLMS onset. A similar association between delta EEG and HR activation has also been observed in connection with arousals without PLM and in the A-phase of CAP (De Carli et al., 2004; Sforza et al., 2000; Halasz et al., 2004). In this context it has been suggested that autonomic arousals without detectable EEG signs (phasic delta-EEG activations and micro-arousals) are different form of arousal response indicating a continuous spectrum in the arousal mechanisms, starting at the brainstem level and progressing to cortical areas (Sforza et al., 2000). From a slightly different point of view Halasz et al. (2004) considered the relationship between vegetative and cortical events as result of a complex interaction between autonomic and cerebral mechanisms which influence each other and does not necessarily imply synchrony or fixed order of activation. Nearly synchronous HR and delta-EEG activation in association with PLMS has been reported by Sforza et al. (2002), while Ferri et al. (2007) visually observed EEG changes preceding the increase in HR. On the other hand, in their recent study, Guggisberg et al. (2007), by applying spectral analysis of HR variability and time frequency analysis of EEG around PLMS, found that autonomic activation started several seconds before movement onset and before EEG changes became evident. They suggested a typical time course beginning with a sympathetic activation followed by sub-cortical activation (mainly expressed by K-complexes and delta bursts) after which a facilitating condition for cortical arousal and PLM occurred. Our results, based on the statistical comparison among onset times of different activations, showed a similar sequence with an early HR increase followed by delta activation preceding PLM, thus supporting the hypothesis of an important role of the autonomic system in starting the complex process in which PLMS occurs.

Table 3
Comparison between NREM and REM sleep as for timing and intensity of activations of different parameters. Anticipation (i.e. the time lasting between the onset of each parameter and the PLM onset), duration and barycentre have been evaluated for NREM and REM sleep (in seconds). Energies are expressed in logarithmic scale. Significant differences ($p < 0.01$) are indicated by an asterisk (*).

	Anticipation (s)		Duration (s)		Energy (Log)		Barycenter (s)	
	NREM	REM	NREM	REM	NREM	REM	NREM	REM
HR	7.30	2.04*	17.83	13.96*	6.41	5.93*	2.05	5.03*
Low delta	3.50	–	10.25	–	4.76	–	0.72	–
High delta	1.30	–	6.45	–	6.07	–	0.60	–
Theta	1.00	0.23	4.52	10.20*	3.40	6.14*	1.19	4.30*
Alpha	0.36	0.18	9.38	12.18*	5.22	6.57*	3.54	5.18*
Sigma	0.27	0.20	10.52	15.20*	6.10	7.12*	4.66	6.18*
Beta	0.36	0.19	16.07	20.50*	7.09	7.71*	6.42	7.68*
Leg Emg	0.05	0.11	10.77	18.05*	11.95	13.45*	2.82	5.45*

A further finding we found was the positive correlation involving leg EMG, fast EEG and HR (in both NREM and REM sleep) and their negative correlation with delta EEG (in NREM sleep). A similar correlation analysis was performed in the mentioned study by Guggisberg et al. (2007), in which they only found a significant inter-subject correlation between autonomic and leg EMG activation and no inter-subject correlation between EEG and autonomic or muscle activities. This difference is probably due to the different patient populations (they studied patients with PLMS and breathing disorders, compared with control subjects, while we selected patients suffering only from insomnia with PLMS in both REM and NREM sleep), to the selection of events (we considered only PLMS rather far each other in order to avoid time-window overlapping, while Guggisberg's study included a larger set of periodic and non-periodic leg movements), and/or to the different way of data processing.

However, the correlations we found between EEG and HR seem in agreement with the common finding that the strength of the autonomic activation progressively increase in association with EEG patterns ranked from phasic delta waves to K-complexes, micro-arousals and arousal with muscle activation (phases of spontaneous transitory activation, according to the classification adopted by Sforza et al. (2000)). With reference to the classification used in the context of CAP (Parrino et al., 2001) the same increase of autonomic activity in association with phase A as ranked from A1 (phasic EEG slow waves) to A2 (mixture of slow and fast EEG activities) and A3 (substantially equivalent to the arousals as defined by the American Sleep Disorders Association (1992)) has been reported.

Interestingly, we found a positive correlation between the strength of fast EEG activities, heart rate and leg EMG, also during REM sleep, where no significant delta-EEG activations were found and CAP sequences were absent, as expected. In such condition, the EEG frequencies typical of REM sleep, namely theta and faster activities, were enhanced, accompanying EMG increase, and the time lasting between the onset of autonomic activation and leg movement was still significantly greater than zero but quite lower than in case of NREM sleep. We can thus only speculate that during REM sleep the interaction of autonomic activation with muscle activity and EEG arousal was more direct for the absence of a significant slow-EEG activation.

An unusual characteristic of our data was that PLM index in REM sleep was only little lower than in NREM sleep: this was probably due to the selection of subjects for a study expressly requesting an important amount of PLM during REM.

Polysomnographic parameters indicated poor sleep in this group of patients, particularly expressed by low sleep efficiency and high wake time after sleep onset, according to the results of a more extensive study (Hornyak et al., 2007) but with even worse values. Also sleep structure, as expressed by the incidence of different sleep stages, suggested a poorer sleep when compared with a control group recorded in the same centre and scored with the same criteria.

Our study confirms that PLMS take place in the context of interacting activations involving the autonomous and central nervous system, where autonomous activation generally precede and probably facilitate movement onset. Associated changes in cerebral activities suggest variations of the arousal level modulating the global response: the presence of a phasic increase of slow EEG waves in NREM sleep is associated to delayed movement onset and lower activations, which may be related to sleep preservation. On the other hand stronger HR and EMG activations are associated with stronger EEG arousals characterized by fast EEG activities leading to sleep fragmentation. Therefore comprehensive analysis of time and intensity of the activation in both NREM and REM sleep and correlation analysis concur in highlighting the different role of autonomic, slow EEG and fast EEG components of arousal reaction and their interaction. The statistical analysis of PLMS and EEG/HR activation onset and intensity applied in this study might be extended to a larger set of periodic and non-periodic limb movement, also considering their overnight distribution, in order to contribute to a better understanding of the relationships between movement, arousal and autonomic system. With our data the possibility to try a comprehensive interpretation, including the role of dopamine system in the control and generation of Cyclic Alternating Pattern and PLM/RLS phenomenology, seems to be too speculative. However, the interpretation suggested by Ferri et al. (2007) giving a role to dopaminergic projections in the balance of descending control on the peripheral excitability could be in this context shareable, hypothesizing a weak top–down hierarchy characterizing all the EEG reflected fluctuations of the arousal level such as spontaneous arousal and CAP A phases.

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