New Approaches to the Study of Periodic Leg Movements During Sleep in Restless Legs Syndrome

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Study Objectives: To describe a new approach for the analysis of quantity, type, and periodicity of the leg motor activity during sleep in patients with restless legs syndrome (RLS) and periodic leg movements (PLM).

Methods: The following parameters were taken into account for LM: duration, amplitude, area under the curve, sleep stage, side, interval, and bilaterality. The analysis of inter-LM intervals was carried out by drawing their distribution graphs. A new index evaluated their periodicity and was validated by means of a Markovian analysis. The differences in inter-LM intervals, LM duration, and area under the curve between normal controls and patients and between the 3 patient subgroups identified on the basis of their periodicity were statistically analyzed.

Setting: N/A

Participants: Sixty-five patients with RLS and periodic LM and 22 young healthy controls.

Measurements and Results: The RLS patients' inter-LM interval distribution graph showed a wide peak with a maximum located at around 15 to 30 seconds and extending from 10 to 90 seconds, not present in controls, and another peak for intervals less than 8 seconds, higher than that of controls. Three patient subgroups were identified with different proportions of these 2 peaks, periodicity, and Markovian parameters. Periodicity was not dependent on the periodic leg movement index. Patients showing the peak mainly at around 15 to 30 seconds tended to show slightly longer and higher area under the curve LM than did the other 2 subgroups.

Conclusions: Our new approach seems to be useful in a new qualitative differentiation among patients with PLM, which is not possible by using the simple PLM index.

Keywords: Restless legs syndrome, periodic leg movements, periodicity index, Markovian analysis

Citation: Ferri R; Zucconi M; Manconi M et al. New approaches to the study of periodic leg movements during sleep in restless legs syndrome. *SLEEP* 2006;29(6):759-769.

INTRODUCTION

THE AMERICAN SLEEP DISORDER ASSOCIATION (ASDA)[1] HAS DEFINED PERIODIC LEG MOVEMENTS (PLM) AS REPETITIVE MUSCLE JERKS LASTING from 0.5 to 5 seconds, separated by an interval ranging from 5 to 90 seconds, with an amplitude of at least 25% of that of the bursts recorded during prerecording calibration, organized in series of 4 or more consecutive leg movements (LM), usually recorded by applying surface electrodes over each anterior tibialis muscle 2 cm apart. Left and right LM have to be scored separately, and classified as 1 bilateral LM when their intermovement interval is shorter than 5 seconds, and as 2 monolateral LM, when it is longer. PLM must be scored according to all sleep stages (PLMS) and in wakefulness (PLMW).[1]

More than 80% of patients affected by restless legs syndrome (RLS) present PLMS.[2-5] However, PLMS can be found in other sleep or neurologic pathologies such as rapid eye movement (REM) behavior disorder,[6] narcolepsy,[7] sleep-related breathing disorders,[8] Parkinson's disease and multiple system atrophy,[9] and also in apparently healthy subjects, especially in the elderly.[8,10] PLMS may be associated with autonomic/electroencephalogram arousals or with awakenings.[11,12] Although the pathologic meaning of PLM is still unclear, a PLMS index (number of PLM per hour of sleep) of 5 or more has been traditionally regarded as clinically significant, if there are accompanying arousals or sleep-depth lightening. However, this threshold value is currently under discussion and revision because PLMS index increases with age in normal subjects, and, probably, a value higher than 5 should be used, at least in the elderly.[8]

The definition and scoring criteria for PLMS and PLMW have not changed since 1993[1] and are substantially based on the work carried out more than 20 years ago by Coleman et al,[13] who collected data from a limited number of subjects using visual analysis of paper polysomnographic recordings. The Atlas and Scoring[1] rules were settled with the purpose of providing recommendations for the correct recording of motor events, the use of standard terminology, and the definition of some common rules to count and evaluate the periodic leg motor activity during sleep. However, with the recent enormous advancement in recording and computer-aided scoring techniques (based on computerized technology), the discover of new pathologies in association with PLMS, different motor patterns, and possible sophisticated methods of signal analysis (e.g., electromyogram [EMG], electroencephalogram, heart rate) have changed our understanding of the impact of periodic motor activity on the macrostructure and microstructure of sleep and its consequences on daytime function.[11,12,14] In particular, the analysis of the EMG signal and of the periodicity of the phenomenon can permit us to evaluate, in different ways, muscle activity in normal and pathologic sleep.

Disclosure Statement

This was not an industry supported study. Drs. Ferri, Zucconi, Manconi, Plazzi, Bruni, and Ferini-Strambi have indicated no financial conflicts of interest.

Submitted for publication September 1, 2005
Accepted for publication February 6, 2006

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For all these reasons, we describe a new methodologic approach in which all leg motor events during sleep are considered for a detailed statistical analysis in order to provide new parameters for the description of the quantity, type, and periodicity of LM during sleep in patients with RLS.

SUBJECTS

Sixty-five consecutive untreated patients affected by idiopathic RLS and PLMS were included in this study (30 men and 35 women, mean age 50.1 years, SD 17.59). In agreement with the International RLS Study Group,[15] the minimal criteria accepted for the diagnosis of RLS were (1) leg restlessness, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs; (2) beginning or worsening of this unpleasant sensation during rest or inactivity such as lying or sitting; (3) partial or total relief of the unpleasant sensations by movement, and (4) worsening or occurrence of the unpleasant sensations in the evening or night, compared to daytime. A mean score of 25.2 (range 13-37)[16] was obtained at the International RLS Study Group rating scale (which was available for only 44 patients). The respiratory pattern during sleep of each patient was assessed in a previous recording by means of oral and nasal airflow (thermistor and/or nasal pressure cannula), thoracic and abdominal respiratory effort (strain gauge), and oxygen saturation (pulse oximetry) (within 1 week) or during this study recording; patients with an apnea-hypopnea index of 5 or greater were not included. Results of a neurologic examination were unremarkable in all patients. Routine blood tests (including serum iron and ferritin levels) and neurophysiologic investigation (EMG and electroneurography of the lower limbs) were also normal. All subjects were free of medication for at least 2 weeks before polysomnography.

Twenty-two young normal controls (12 men and 10 women, mean age 30.9 years, SD 6.18) were also included in the study in order to evaluate the features of LM activity in healthy subjects and to avoid normal subjects with high values of PLMS index, which are frequently found in individuals older than 40 years, even in the absence of any sleep complaint.[17] Control subjects were screened to exclude those with any current or prior symptoms suggestive of RLS by using the same International RLS Study Group[15] minimal criteria for the diagnosis of RLS and had to be in general good health; they were excluded from the study if any of the following were present: diagnosis of any other significant sleep disorder; major mental illness, including any indications of cognitive problems as determined by history; or any history of neuroleptic-induced akathisia or use of any neuroleptic in the past year.

In order to carry out a reliable comparison with controls, RLS subjects were further subdivided into 2 age subgroups: 22 "young" (13 men and 9 women, mean age 29.0 years, SD 8.62) and 43 "old" patients (17 men and 26 women, mean age 60.8 years, SD 9.10). The comparison between normal controls and RLS patients was carried out using only the young patient subgroup whereas both patient subgroups were used to analyze age-related changes in PLM parameters.

METHODS AND RESULTS

Polygraphic Sleep Recording

Each subject underwent a polysomnographic full-night recording, after an adaptation night, carried out in a standard sound-attenuated (noise level to a maximum of 30 dB) sleep laboratory. Subjects were not allowed to have beverages containing caffeine during the afternoon preceding the recording and were allowed to sleep until their spontaneous awakening in the morning.

The following parameters were included in the polysomnographic study: electroencephalogram (at least 3 channels, 1 frontal, 1 central and 1 occipital, referred to the contralateral earlobe); electrooculogram (electrodes placed 1 cm above the right outer cantus and 1 cm below the left outer cantus and referred to A1), EMG of the submentalis muscle, EMG of the right and left tibialis anterior muscles (bipolar derivations with 2 electrodes placed 3 cm apart on the belly of the anterior tibialis muscle of each leg impedance was kept less than 10 KΩ, according to the ASDA[1] scoring criteria); and electrocardiogram (1 derivation). Sleep signals were sampled at 200 Hz and stored on hard disk in European data format (see Kemp et al[18] for details) for further analysis. EMG signals, in particular, were digitally band-pass filtered at 10 to 100 Hz, with a notch filter at 50 Hz.

At the beginning of each session and before the start of recording, the sleep technician checked that the amplitude of the EMG signal from the 2 tibialis anterior muscles was below 2 μV at rest and exceeded 7 to 10 μV for small voluntary flexions of the foot. The EMG amplitude at maximal deflection was also measured for the application of the ASDA[1] scoring criteria.

Sleep Scoring and Detection of LM

Sleep stages were scored following standard criteria[19] on 30-second epochs by means of the sleep-analysis software Hypnolab 1.2 (SWS Soft, Italy). LM during sleep were first detected by the same software that allows their computer-assisted detection. With this software, the detection is performed by means of a human-supervised automatic approach controlled by the scorer. The performances of this system have been evaluated and validated,[20] but, for this study, 1 scorer visually edited the detections proposed by the automatic analysis before the computation of the various parameters, which were automatically generated by the same software adopting the ASDA[1] criteria. In particular, a total LM Index was calculated to represent the total number of LM per hour of sleep while the PLMS index was calculated as the number of LM included in a series of 4 of more, separated by more than 5 and less than 90 seconds, per hour of sleep.

Table 1 shows the comparison between the sleep-scoring parameters found in the young patient subgroup and normal controls or old patients with RLS and PLM. This comparison discloses many expected differences between the 2 young groups under analysis. In particular, patients showed significantly higher values of time in bed, sleep-period time, first REM latency, number of awakenings per hour, and percentage of wakefulness after sleep onset and of sleep stage 1 and significantly smaller values of sleep efficiency and slow-wave sleep. On the contrary, the comparison between young and old patients with RLS showed only few significant differences: reduced sleep efficiency and REM sleep percentage and increased amount of wakefulness after sleep onset.

Table 2 shows the same type of comparison for the classic PLM parameters. Also, in this case, as expected, the young patient group showed values higher than those of controls for almost all the parameters considered, and, in particular, the average

Table 1—Comparison Between the Sleep-Scoring Parameters Found in Young Normal Controls and in the 2 Age Subgroups of Patients with RLS

	1. Controls n = 22		2. Young RLS n = 22		3. Old RLS n = 43		Kruskal-Wallis ANOVA	Mann-Whitney test p value <	
	Mean	SD	Mean	SD	Mean	SD	p value <	1 vs. 2	2 vs. 3
TIB, min	428.8	45.25	517.9	95.79	517.0	87.82	.00001	.00015	NS
SPT, min	412.0	47.74	488.4	101.98	478.3	96.05	.0004	.003	NS
TST, min	393.1	41.57	434.9	104.02	380.0	97.64	NS	~	~
SOL, min	13.6	11.61	19.0	26.75	29.2	34.73	NS		
FRL, min	63.5	28.31	113.4	58.25	121.6	68.30	.0002	.002	NS
SS/h	10.8	3.10	14.8	6.79	11.8	3.99	NS	~	~
AWN, no./h	1.9	1.18	5.7	4.71	5.1	2.36	.0001	.001	NS
MT, no./h	1.0	0.59	0.7	1.11	0.4	0.78	.0006	.025	NS
SE, %	91.8	4.06	83.8	11.20	72.9	14.45	.00001	.002	.001
WASO, min	4.4	3.33	11.1	8.59	21.4	12.54	.00001	.0015	.002
Sleep stage, %									
1	1.4	1.36	8.3	6.59	7.8	5.12	.00001	.00001	NS
2	45.6	7.27	45.4	10.33	42.1	10.15	NS	~	~
SWS	24.2	6.13	15.0	5.59	15.1	7.69	.00001	.00003	NS
REM	24.3	5.39	20.2	7.37	13.7	4.64	.00001	NS	.00008

RLS refers to restless legs syndrome; TIB, time in bed; SPT, sleep period time; TST, total sleep time; SOL, sleep-onset latency; FRL, first rapid eye movement (REM) latency; SS/h, stage shifts per hour; AWN/h, awakenings per hour; MT/h, movement time events per hour; SE, sleep efficiency; WASO, wakefulness after sleep onset; 1, 2, sleep stages 1 and 2; SWS, slow-wave sleep; ANOVA, analysis of variance.

Table 2—Comparison Between the Classic PLM Parameters Found in Young Normal Controls and in the 2 Age Subgroups of Patients with RLS and PLM

	1. Controls n = 22		2. Young RLS n = 22		3. Old RLS n = 43		Kruskal-Wallis ANOVA	Mann-Whitney test p value <	
	Mean	SD	Mean	SD	Mean	SD	p value <	1 vs. 2	2 vs. 3
Total LM, index	8.3	3.98	40.6	16.66	44.0	19.99	.00001	.00001	NS
PLM, index	2.3	2.18	30.4	17.45	36.7	20.26	.00001	.00001	NS
Isolated LM, index	5.9	2.44	10.2	3.77	7.3	3.45	.0002	.00001	.002
Monolateral, n	20.0	16.96	111.1	56.72	103.8	68.31	.00001	.00008	NS
Bilateral, n	35.5	18.73	171.6	79.81	170.6	120.77	.00001	.00001	NS
PLM sequences, n	2.5	2.42	16.6	8.51	12.0	6.25	.00001	.00001	.03
PLM sequence duration, seconds	4.8	12.16	75.7	85.00	117.4	203.20	.00001	.00001	NS
PLM duration, seconds	2.7	1.53	2.5	0.73	2.5	0.72	NS	~	~
Isolated LM duration, seconds	2.8	1.34	2.7	0.80	2.8	1.06	NS		

PLM refers to periodic limb movements; RLS, restless legs syndrome; LM, leg movements.

PLM index was 30.4 in patients and 2.3 in normal controls. Only the mean duration of PLM and of isolated LM was practically the same in the 2 groups. On the contrary, very few differences were found between young and old patients with RLS involving the index of isolated LM and the number of PLM sequences, which were both slightly higher in the young patients.

New Parameters for the Analysis of LM During Sleep

Duration—After the calculation of the classic parameters based on the ASDA[1] criteria, we modified our analysis ranges in order to include all movements lasting 0.5 to 15 seconds; similar to the ASDA[1] report, this choice was based on the fact that EMG bursts shorter than 0.5 seconds are usually associated with muscle activities outside of the scope of the present study, such as short sleep starts, REM-sleep-related activity, phasic EMG activity, and fragmentary myoclonus.[21–27] Moreover, we also included LM with a duration up to 15 seconds because movements longer than 15 seconds during sleep usually characterize the so-called movement time stage.[19]

Amplitude—As introduced above, the amplitude of the EMG signal from the 2 anterior tibialis muscles was below 2 μV at rest and exceeded 7 to 10 μV for small voluntary flexions of the foot. LM included in this study had an initial amplitude of at least 10 μV; the ending point was detected by finding where, after the detection of the start point, the average amplitude of the signal, calculated over a sliding epoch of 0.5 seconds, returned to a value below 2 μV. All these values were calculated on the rectified EMG signals.[20]

Area Under the Curve—LM are characterized by a variable EMG level; we calculated the area under the curve (in μV/s) in order to obtain a more reliable quantity that takes into account the variable amplitude and the duration of each LM.

Sleep stage—Sleep stage in which each LM starts.

Side—Right or left leg.

Start and ending time—These 2 values were used for the calculation of the 2 intervals described below.

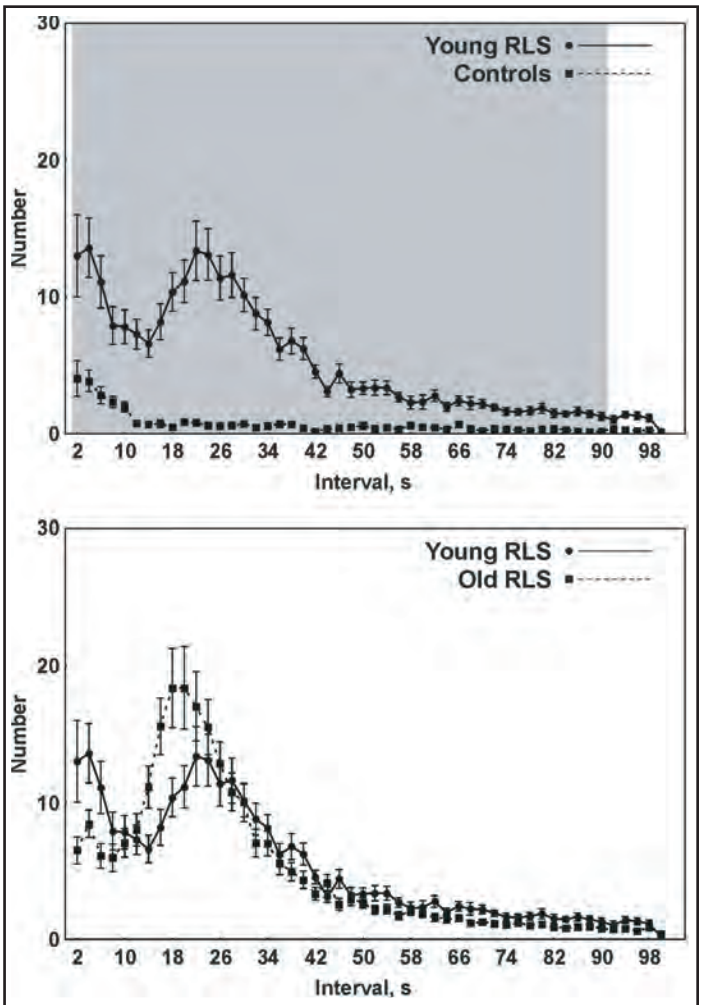

Figure 1—Top panel: Comparison between the distribution of inter-leg movement (LM) intervals in young normal controls and in the group of young patients with restless legs syndrome (RLS) and periodic limb movements (PLM); values are shown as mean and SEM (whiskers). The grey-shaded area shows points for which a significant difference was found between these 2 groups during statistical analysis. Bottom panel: Comparison between the distribution of inter-LM intervals in young and old patients with RLS and PLM; values are shown as mean and SEM (whiskers).

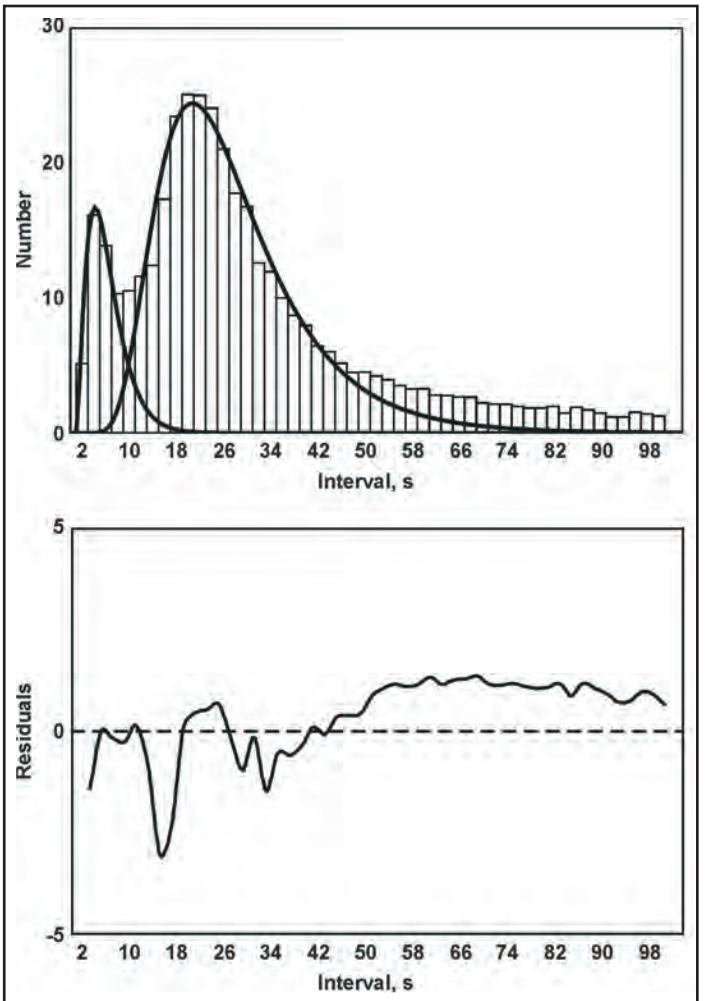

Figure 2—Top Panel: Distribution of inter-leg movement (LM) intervals in all patients with restless legs syndrome (RLS) and periodic limb movements (PLM) (bar histogram) and the 2-lognormal distributions obtained by fitting these data (solid lines). Bottom panel: Residuals obtained by subtracting the values expected with our 2-lognormal distributions from those observed (bar histogram).

Interval 1—This interval was defined as the time between the onsets of 2 subsequent LM and was used for the evaluation of their periodicity (see below).

Interval 2—Defined as the time between the end of a LM and the onset of the following LM; this interval was used for the separation of different LM intervening in the same leg on or the contralateral leg.

Minimum interval between 2 different LM—We applied a time resolution of 0.5 seconds for the detection of the presence of movement (see above); we then applied the same time resolution for the detection of the absence of movement. For this reason, the minimum interval between different LM (Interval 2) was set to 0.5 seconds.

Bilateral/monolateral movements—Monolateral LM were defined as EMG bursts involving only 1 leg and separated by at least 0.5 seconds from any other LM; bilateral LM were defined as 2 EMG bursts on the 2 legs overlapping or separated (Interval 2) by less than 0.5 seconds. Bilateral movements could include 2 or more monolateral movements (Interval 2) separated by less than 0.5 seconds from each other.

Combined leg analysis—Periodicity (see below) was evaluated combining the LM detected in both legs, and bilateral movements were counted as one.

Analysis of Inter-LM intervals

The top panel of figure 1 reports the comparison between the distribution of inter-LM intervals in young normal controls and in the group of young patients with RLS and PLM. In this analysis, all inter-LM intervals were counted, in each subject, for 2-second classes ($0.5 < \text{interval} \leq 2$, $2 < \text{interval} \leq 4$, $4 < \text{interval} \leq 6$,…. $98 < \text{interval} \leq 100$). The grey-shaded area shows points for which a significant difference was found between these 2 groups during statistical analysis performed by means of the nonparametric Mann-Whitney test; in order to take into account the multiple comparisons performed, the Bonferroni correction was used for the level of significance and differences were considered as significant when they reached $p < .001$ (.05/50).

Interestingly, the 2 graphs are significantly different for interval classes up to 90 seconds, the same interval that was indicated

by the ASDA as the maximum length for intervals between PLM.[1] The normal control graph shows its maximum value for the shortest intervals (0.5 < interval ≤ 2 seconds), which decreases rapidly from this class to that relative to intervals 8 < interval ≤ 10 seconds; interval classes above this value are very close to 0. On the contrary, the young patient graph shows a clearly bimodal distribution of intervals with 1 peak at 2 < interval ≤ 4 seconds and another at around 20 < interval ≤ 22 seconds; this peak decreases progressively up to 88 < interval ≤ 90 seconds and, after this class, the patient graph is not distinguishable from that of normal controls. The bimodal distribution of this graph seems to indicate the presence of 2 categories of inter-LM intervals; the first probably representing an exaggeration of the peak seen in normal controls, and the second representing the occurrence of PLM.

The bottom panel of Figure 1 shows the same type of comparison carried out between the distribution of inter-LM intervals in young and old patients with RLS and PLM. Although some differences might be suspected by visual analysis, the graphs were not statistically different in any of their points. Because of this and of the results obtained from sleep structure and from the application of the classic PLM parameters, we decided to use the group of patients as a whole in the following analyses described below.

Modeling the Inter-LM Interval Distribution and Development of a New Index for LM Periodicity

In order to model the bimodal distribution of inter-LM intervals found in our patients, we performed a distribution mixture analysis; a variety of different distributions were tested, and the best fit was found with 2-lognormal distributions (see Figure 2, top panel). The first distribution on the left accounts for the first peak in the graph and has a shape parameter of approximately 1, whereas the second accurately describes the second peak up to approximately 60 seconds and has a shape parameter of approximately 0.5. The 2 fitted distributions intersect at around 10 seconds. The bottom panel shows the residuals obtained by subtracting the values expected with our 2-lognormal distributions from those observed (bar histogram); they are low and always lower than 3. The χ^2 test was computed, which showed a significant difference between the fitted and the observed distributions if all values up to 90 or 100 seconds were considered; on the contrary, the fitting passed the goodness-of-fit test only if values up to 60 seconds were included.

The clear bimodal distribution of inter-LM intervals in our patients poses 2 main problems: (1) it is not possible to statistically describe this type of distribution in terms of mean and standard deviation; (2) the notch between the 2 peaks in the graph occurs in correspondence to the point at which the normal control graph approaches values of 0 (10 seconds), therefore, if the left peak in the patient graph represents an exaggeration of the normal peak, only inter-LM intervals longer than this value should be considered as belonging to PLM.

However, periodicity cannot be extrapolated from only the analysis of this graph because it represents only the count of intervals in a class; for the evaluation of periodicity, we retained some features of the approach suggested by the ASDA criteria[1] for PLM and counted all intervals in the series of each subject with a length of 10 < interval ≤ 90 seconds that were preceded and followed by another interval with the same length; this is equivalent to a miniseries of 4 LM all separated by intervals with a length of 10 < interval ≤ 90 seconds. Subsequently, the number of intervals with these characteristics was subdivided by the total number of intervals; we will refer to this ratio as the Periodicity Index, which is defined by the formula:

Periodicity Index = no. of sequences of 3 inter-LM intervals 10 < interval ≤ 90 seconds / total number of inter-LM intervals

This index can vary between 0 (absence of periodicity, with none of the intervals having a length between 10 and 90 seconds) to 1 (complete periodicity, with all intervals having a length between 10 and 90 seconds). Periodicity Index is independent on the absolute number of LM recorded, and it was calculated for all the subjects included in this study. The mean value ± SD of Periodicity Index was 0.239 ± 0.225 in normal controls and 0.668 ± 0.184 in the whole group of RLS patients ($p < .000001$ Mann-Whitney test); Periodicity Index was 0.597 ± 0.188 in young RLS patients versus 0.704 ± 0.174 in old RLS patients ($p < .035$ Mann-Whitney test).

The values of Periodicity Index were used, in the whole group of patients, for different correlations with their age, their score at the RLS rating scale,[15] and the classic PLM parameters shown before, by means of the nonparametric Spearman rank-order correlation. Table 3 shows the values of correlations found and their statistical significance, which was only reached for the index of isolated LM, number of bilateral LM, and number of PLM sequences—all negatively correlated with the Periodicity Index. Interestingly, the correlation between Periodicity Index and age, even if positive, did not reach statistical significance at this test.

Subsequently, even if the Periodicity Index seems to be distributed in a continuous fashion in patients, we tried to take into account its important variability seen in the different patients by establishing 3 arbitrary ranges of Periodicity Index , with the aim of defining 3 patients subgroups that might be of practical use in future studies. These 3 ranges were indicated as PLM1 (Periodicity Index ≥ 0.75), PLM2 (Periodicity Index 0.50 ≤ Periodicity Index < 0.75), and PLM3 (Periodicity Index < 0.50). The rationale behind this choice is that subjects belonging to the PLM1 subgroup have the vast majority of their LM included into PLM sequences; on the contrary, those in the PLM3 subgroup have a preponderance of nonperiodic LM, with those in the PLM2 subgroups having an intermediate situation. In other words, subjects

Table 3—Correlation between Periodicity Index and Age or the Classic PLM Parameters Found in the Whole Group of Patients

	Spearman rank order correlation	p <
Age, y	0.197	NS
RLS rating scale, score	0.030	NS
Total LM, index	-0.070	NS
PLM, index	0.038	NS
Isolated LM, index	-0.532	.00001
Monolateral, no.	0.042	NS
Bilateral, no.	-0.367	.01
PLM sequences, no.	-0.509	.0001
PLM sequence duration, sec	0.167	NS
PLM duration, sec	0.108	NS
Isolated LM duration, sec	0.155	NS

PLM refers to periodic limb movements; RLS, restless legs syndrome; LM, leg movements.

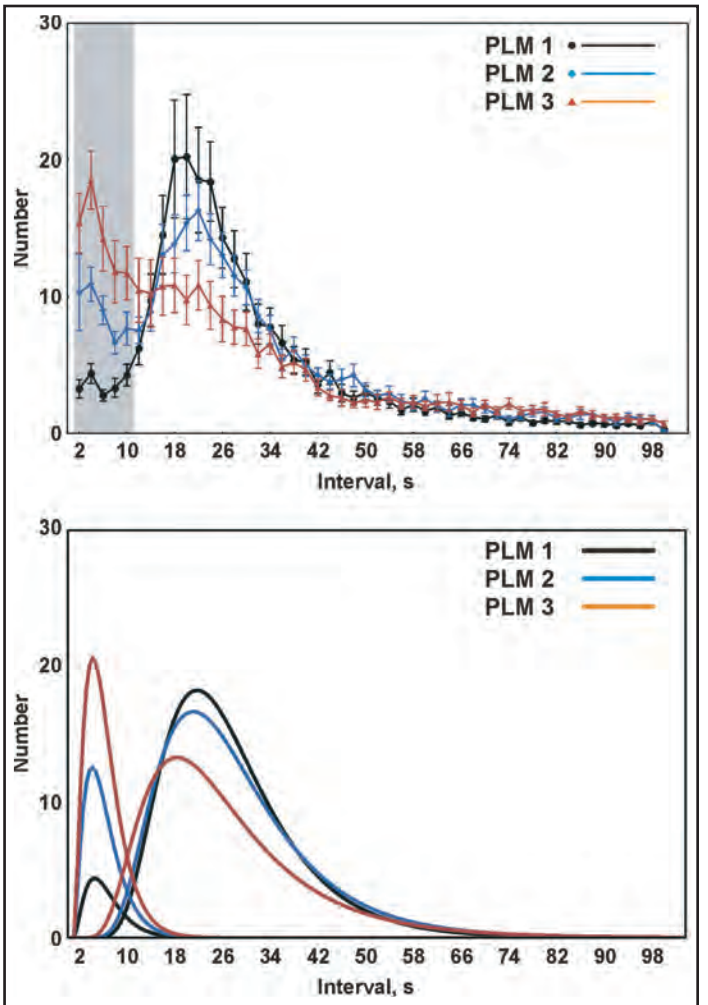

Figure 3—Top panel: comparison between the distribution of inter-leg movement (LM) intervals in the 3 periodic limb movements (PLM) patient subgroups; the grey-shaded areas show points for which a significant difference was found between these groups during statistical analysis. Bottom panel: lognormal distributions obtained by fitting the data shown in the top panel (colored solid lines).

belonging to the PLM1 subgroup tend to have their inter-LM interval distribution graph dominated by a single peak in the zone 10 < interval ≤ 90 seconds; on the contrary, patients belonging to the PLM3 subgroup show a main peak in the zone interval ≤ 10 seconds, and subjects in the PLM2 subgroup show the bimodal distribution seen above. The subgroups identified were formed by PLM1 = 27 patients (55.4 ± 13.19 years); PLM2 = 22 patients (46.8 ± 19.91 years); PLM3 = 16 patients (45.5 ± 19.37 years); these ages were not significantly different at the nonparametric Kruskal-Wallis analysis of variance.

The top panel of Figure 3 reports the comparison between the distribution of inter-LM intervals in the 3 PLM patient subgroups; the grey-shaded area shows points for which a significant difference was found between these groups during statistical analysis (Bonferroni adjusted p at the nonparametric Kruskal-Wallis analysis of variance, see above). The bottom panel of the same figure shows the distribution modeling for each of the observed distributions, obtained similarly to that in Figure 2. These graphs show that the 3 subgroups differ mainly for the amount of inter-LM intervals ≤ 10 seconds.

Figure 4 shows the comparison between Total LM index, Periodicity Index, and PLM index in young normal controls and in the 3 PLM patient subgroups. The statistical significance of this comparison for Total LM index and PLM index is reported in Table 4. For the Periodicity Index, no statistical test was performed because the 3 subgroups were arbitrarily arranged on the basis of their difference for this parameter. This figure shows that PLM index and Total LM index cannot separate the 3 PLM subgroups identified by means of our Periodicity Index; conversely, Periodicity Index is not dependent on the absolute values of these parameters. Also, Periodicity Index clearly distinguishes between patients and normal controls, even if some of the latter tend to show values that overlap with those of the PLM2 and PLM3 subgroups.

Sleep Staging and "Classic" PLM Parameters in our PLM Subgroups

The 3 PLM subgroups did not differ significantly for any of the sleep-scoring parameters considered. Moreover the comparison between the classic PLM parameters found in the 3 PLM patient subgroups, reported in Table 4, shows that the index of isolated LM was always higher in the PLM3 subgroup than in the other 2 subgroups, with PLM1 always showing the smallest number.

Table 4—Comparison Between the Classic PLM Parameters Found in the 3 Subgroups of Patients With PLM

	PLM 1 n = 27		PLM 2 n = 22		PLM 3 n = 16		Kruskal-Wallis ANOVA
	Mean	SD	Mean	SD	Mean	SD	p value <
Age, y	55.4	13.19	46.8	19.91	45.5	19.37	NS
RLS rating scale, score	25.5	5.88	24.9	6.56	25.0	4.47	NS
Total LM, index	43.1	21.97	44.3	17.20	40.4	16.07	NS
PLM, index	36.8	22.23	36.1	18.24	28.7	15.62	NS
Isolated LM, index	6.3	2.24	8.3	3.75	11.7	3.72	.0001
Monolateral, no.	178.5	129.44	184.6	102.07	139.4	68.50	NS
Bilateral, no,	88.5	66.08	106.2	57.85	136.3	61.95	.035
PLM sequences, no,	9.6	4.16	15.0	7.28	18.3	8.51	.0006
PLM sequence duration, sec	149.7	242.16	99.5	105.48	30.3	24.44	NS
PLM duration, sec	2.7	0.82	2.3	0.66	2.5	0.52	NS
Isolated LM duration, sec	2.9	1.03	2.6	0.95	2.6	0.94	NS

PLM refers to periodic limb movements; RLS, restless legs syndrome; LM, leg movements; ANOVA, analysis of variance.

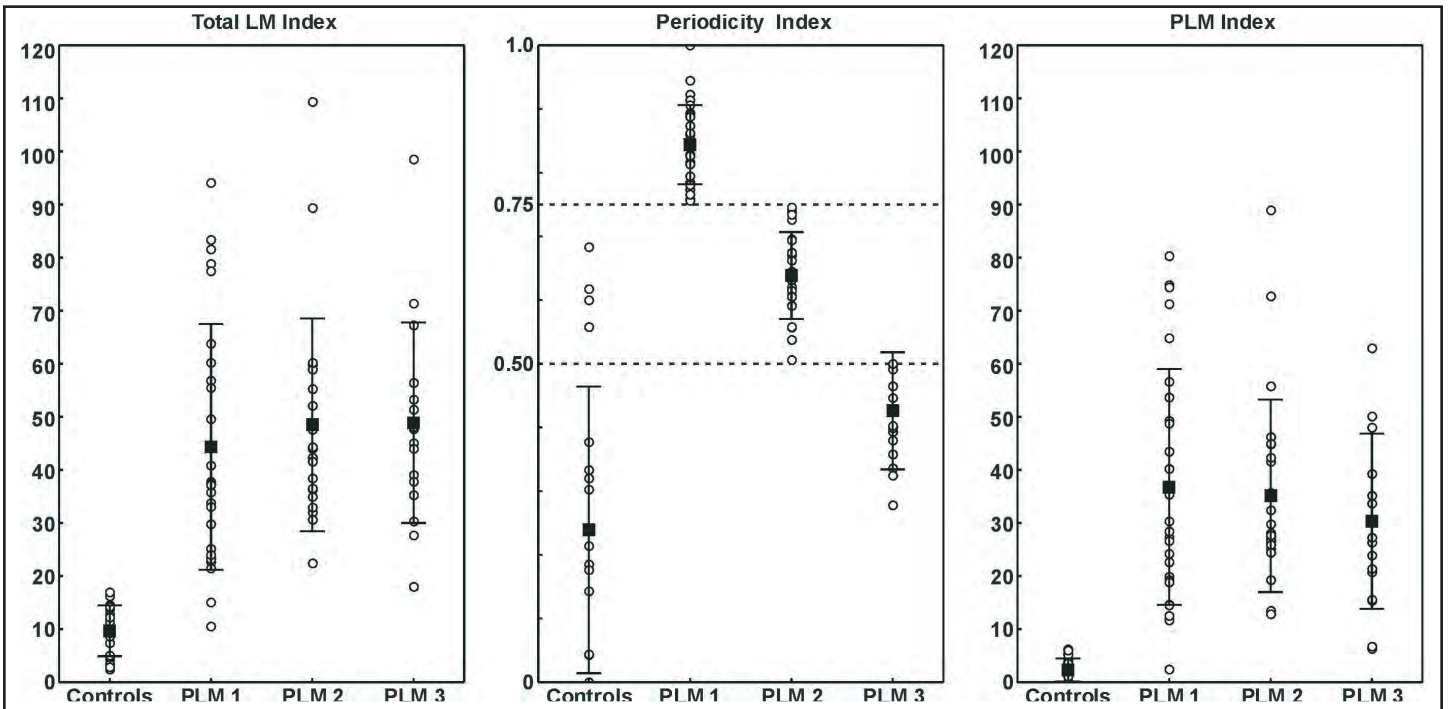

Figure 4—Comparison between Total Leg Movement (LM) Index, Periodicity Index, and Periodic Limb Movements (PLM) Index in young normal controls and in the 3 subgroups of patients with PLM. The statistical significance of this comparison for Total LM Index and PLM Index is reported in Table 2. For the Periodicity Index, no statistical test was performed because the 3 subgroups were arranged on the basis of their difference for this parameter.

As seen before, the total LM index and the PLM index are very similar in the 3 subgroups; only the number of PLM sequences is higher in the PLM1 subgroup than in the other 2 subgroups. The average score on the International RLS Study Group rating scale[16] was not different in the 3 PLM subgroups.

Table 5—Example of the Markov Chain Analysis of PLM Intervals in a Patient with RLS and PLM

	Zero-memory Markov model		
	Interval duration, s	**Events, no.**	**Probability**
State 1	< 10	31	.119
State 2	≥ 10 ≤90	208	.800
State 3	>90	21	.081

H0 = 0.917 bits/state

	First-order Markov model Transition probability matrix		
	State 1	**State 2**	**State 3**
State 1	0.129	0.839	.032
State 2	0.130	0.837	.034
State 3	0.000	0.381	.524

H1 = 0.782 bits/state
Dependency index = 0.147
Shuffled Data Statistics
Z-score = 45.519 ($p < .00001$)

The top rows show the calculation of the entropy of the zero-memory model (H0); the middle rows report the transition probability matrix of the first-order Markov model, the relative entropy value (H1), and the Dependency index. The last rows display the results of the statistical approach; the Z-score indicates a significant difference between the observed time structure and that expected for a random process with the same probability of occurrence of each state. RLS refers to RLS; PLM, periodic limb movements.

PLM Time Structure Analysis

Markovian Analysis

In order to confirm the validity of our new Periodicity Index, we analyzed the structure of the LM sequence, in each subject studied, by means of an approach known to be able to characterize the time dependency (also a feature of periodicity) of time series, ie the analysis of Markov chains.[28] A Markov chain is a sequence of values (states) that have probabilities at a time interval that depend upon the value at the previous time. The controlling factor in a Markov chain is the transition probability, which is a conditional probability for the system to go to a particular new state, given the current state of the system. From these probabilities, the entropy of the system can be computed. The basic concept of entropy in information theory deals with how much randomness is in a signal or in a sequence of events. Alternatively, the entropy gives a measure of how much information is carried by the signal.

In this study, for each subject included, the length of each interval between subsequent LM was assigned to 3 states as follows: State 1 (interval ≤ 10 seconds), State 2 (10 < interval ≤ 90 seconds), and State 3 (interval > 90 seconds). First, we calculated the unconditional probability of occurrence of each state from which we obtained the zero-memory Markov model entropy (H0); in other words, we considered each state as occurring following its own intrinsic probability, not conditioned by the previous state.

Subsequently, a 3 × 3 matrix was obtained; the 9 entries in this matrix were the probabilities of transition from a given state to the next state, in successive interval occurrences. For example, if the transition from State 1 to State 3 occurred 7 times in N possible transitions from State 1 to any other State (including State 1), then the transition probability in cell [1,3] of the matrix (i.e.,

cell in the first row and third column of the matrix) was given the value 7/N. Of course, the sum of all probability entries in a row of the matrix (e.g., the contents of cells [2,1], [2,2], and [2,3]) had to be 1. This transition probability (or conditional probability) matrix (TPM) is the state TPM of the Markov chain theory, and it has been used in a similar way to quantify the time structure of other sequences of events recorded during sleep.[29-32] For a reliable estimation of transition probabilities, a number of transitions equal to at least 8 times the number of matrix entries is needed.[33] Since the average total number of LM available for each patient was 280, the use of 3 × 3 matrixes (9 matrix entries) can be considered as reliable in this group; on the contrary, a mean total number of LM of 65 was available for normal controls. Each matrix served for the computation of the first-order Markov model entropy (H1); if the value of H1 is lower than that of H0, one can suppose that first-order relationships exist between the states of the system, i.e., each state does not occur following only its own probability, but is influenced by the value of the state preceding it. The degree of this influence can be described by means of the Dependency Index (= H0-H1/H0), which can range from 0 (lack of first-order interdependencies) to 1 (complete first-order dependency).

Statistical Analysis of TPMs

In order to test further the null hypothesis that the TPM were generated by a random process, we randomly shuffled the states of each sequence and recalculated H0, H1, and the Dependency Index 25 times. Random shuffling destroys all interdependencies in the sequence, and the values obtained from these shuffled-state sequences can be used for the statistical validation of each single TPM. This validation was performed by calculating the Z-score between the value of the Dependency Index obtained from the original state sequence and the 25 values obtained from the shuffled data. Table 5 shows an example of the Markov chain analysis of PLM intervals in 1 patient with RLS and PLM. The top rows show the calculation of the entropy of the zero-memory model (H0), whereas the middle rows report the TPM of the first-order Markov model, the relative entropy value (H1) and the Dependency Index. The last rows display the results of the statistical approach; the Z-score indicates a significant difference between the observed time structure and that expected for a random process with the same probability of occurrence of each state. This analysis was statistically significant in all patients with RLS; on the contrary, only 2 normal controls showed values of the Dependency Index significantly different from those expected for a random process. This was due to the low number of LM in normal subjects, which made the computation of the Markov parameters weak; for this reason, the values obtained in normal controls could not be considered as reliable and were excluded from the following statistical analysis. This might also mean that a certain minimum number of LM are needed for a correct evaluation of the Periodicity Index; this was not assessed in the present study and needs to be clarified in the future.

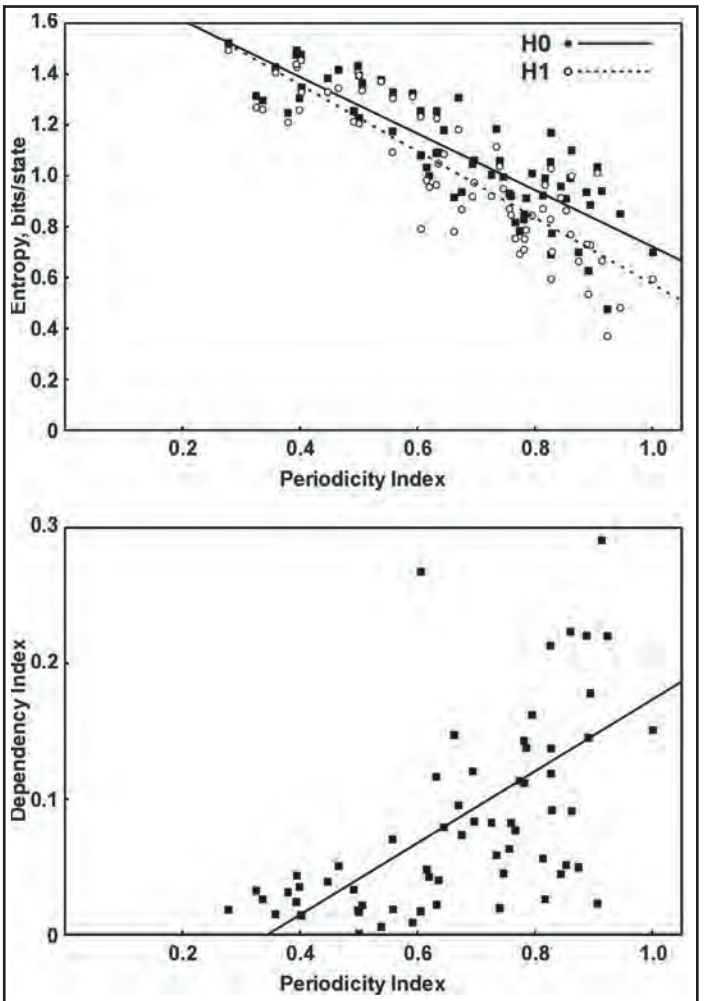

Figure 5—Correlation between Periodicity Index and H0, H1 (top panel), and Dependency Index (bottom panel), in the whole group of patients with restless legs syndrome; the regression lines are also shown.

Figure 5 shows the correlation between the Periodicity Index and H0, H1, and the Dependency Index, in the whole group of patients with RLS and was calculated by means of the nonparametric Spearman rank order test, the results of which were all highly significant (H0 = -0.837, $p < .00001$; H1 = -0.856, $p < .00001$; Dependency Index = 0.695, $p < .00001$). Both measures of entropy decrease with an increasing Periodicity Index, while the Dependency Index has a significant positive correlation with the Periodicity Index. In Table 6, the comparison between the results obtained from the Markov chain analysis in the 3 PLM patient subgroups is shown. The values of entropy show smallest values

Table 6—Comparison Between the Results Obtained From the Markov Chain Analysis in the 3 PLM Patient Subgroups

	1. PLM 1 n = 27		2. PLM 2 n = 22		3. PLM 3 n = 16		Kruskal-Wallis ANOVA	Mann-Whitney test p <		
	Mean	SD	Mean	SD	Mean	SD	p <	1 vs 2	1 vs 3	2 vs 3
H0, bits/state	0.881	0.1529	1.140	0.1427	1.377	0.0937	.00001	.000001	.000001	.000035
H1, bits/state	0.763	0.1615	1.067	0.1750	1.341	0.0948	.00001	.000001	.000001	.00003
Dependency index	0.136	0.0906	0.068	0.0593	0.026	0.0130	.00001	.0015	.000001	.006

PLM refers to periodic limb movements; ANOVA, analysis of variance; H0, entropy of the zero-memory model; H1, relative entropy value.

for the PLM1 subgroup and their highest values for the PLM3 subgroup; the differences observed between the subgroups were statistically significant. Also, the Dependency Index obtained in the PLM1 subgroup was significantly higher than that obtained in the other subgroups. These analyses confirm that our Periodicity Index is able to pick the time structure of the LM activity in patients with RLS and PLM and correlates negatively with the degree of randomness in the time series.

Analysis of LM Duration

Patients showed a higher number of LM than did controls for durations up to 11 seconds; however, the distribution was substantially the same with a progressively decreasing number of LM from shorter to longer durations. The PLM1 subgroup showed a statistically significant lower number of LM with short duration (0.5-1 seconds) than the other 2 subgroups. Thus, in this case, the distributions were slightly different among the 3 PLM subgroups.

Analysis of LM AUC

RLS patients showed a higher number of LM than did normal controls for almost all AUC values; however, the distribution was substantially the same, with a progressively decreasing number of LM from smaller to bigger AUCs. In this case, The PLM1 subgroup showed a statistically significant lower number of LM with small AUC (≤ 10 μV/s) than the other 2. Also, in this case, the distributions seemed to be different among the 3 PLM subgroups.

DISCUSSION

Since the discovery of involuntary pseudoperiodic leg jerks during sleep in patients affected by RLS, it was immediately clear that their polysomnographic characterization could be used as a possible fundamental objective neurophysiologic marker in RLS diagnosis and in quantification of its severity. For this purpose, after a visual-manual detection of LM on paper polysomnographic studies of patients presenting with PLMS, Coleman et al[13] described this motor phenomenon in terms of duration, amplitude, periodicity, and symmetry, creating the basis of the scoring criteria accepted later by the ASDA[1] and commonly used today by all sleep labs. It is well know that the diagnostic value of PLM in patients with RLS is sustained by a high sensitivity, but, at the same time, it is also reduced by a low specificity, since PLM are also a common finding in other sleep disorders and in healthy elderly subjects. On the other hand, the value of PLM as a marker of RLS symptom severity seems to be supported by recent investigations in which a significant relationship between PLMS index and score on the International RLS Study Group rating scale[16] has been demonstrated.[34] Although several studies conducted in the last decades have focused on PLM, the real pathophysiologic meaning of this polysomnographic event remains unclear, and PLM still represents one of the most intriguing mysteries in sleep medicine.

In this study, we applied a new method of analyzing LM during sleep in a large group of patients with RLS and control subjects, with the intent of improving our knowledge about PLM by means of a detailed characterization of their features. The main difference between our approach and the original method used by Coleman et al[13] is that our approach is empirical and theirs was intuitive and rational; other important differences are represented by the larger number of subjects analyzed, the digital versus the analog recording, the automatic validated detection of LM[20] associated with a manual visual correction versus only manual detection, and the use of a new statistical approach to describe the PLM phenomenon and to enhance the significant differences between RLS and normal subjects. Additional aims of the study were to verify if, by using the standard criteria, we lose important pieces of information and to ascertain the presence of possible different PLM patterns in the RLS population.

In our investigation, we took into consideration a wide spectrum of phasic sleep activities of the anterior tibialis muscles, including nonperiodic LM and LM with a duration up to 15 seconds. Afterward, each parameter (duration, AUC, and inter-LM interval) was compared between RLS and control subjects to evaluate possible quantitative and qualitative differences. As expected, using the standard ASDA criteria,[1] patients with RLS differed from control subjects in that they had a higher total and sleep-phase-specific PLM index and a worse sleep quality.

First of all, it is important to point out that, for the 3 main parameters considered in this study (interval, duration, and AUC), a non-Gaussian distribution was found in both normal controls and patients with RLS. This implies that the use of means and standard deviations is not adequate to statistically describe these parameters and to statistically test differences between groups or conditions. We strongly suggest the use of distribution graphs for the statistical characterization of these parameters.

The average LM duration was found to be not different between controls and patients; the distribution histograms also had a similar aspect. On the other hand, this approach showed that polysomnograms of patients had a significantly higher number of LM, as compared with normal controls, for durations up to 11 seconds. This means that the duration criteria established by ASDA,[1] considering only movements ranging from 0.5 to 5 seconds, might be too restrictive and might exclude movements potentially belonging to the same pathophysiologic family of PLM. Other authors have already suggested broadening the duration criterion to include movements exceeding 5 seconds, especially during non-REM sleep stage 1 or when PLM are associated with arousals. For PLMW, Michaud et al[35] proposed to also include movements lasting up to 10 seconds. We did not measure PLMW with either the classic scoring criteria or with our new approach; however, in the future, the analysis of LM features during presleep or intrasleep wakefulness should also be refined because PLMW show a good correlation with the severity of the clinical syndrome in patients with RLS.[36]

Considering the new parameter of the AUC, significant quantitative differences between normal and RLS groups have been found for almost all values considered. This result suggests that no restrictive upper limits should be applied to PLM on the basis of this parameter; this is related to the fact that EMG is an uncalibrated signal, which can show large interindividual and intraindividual changes, even during the same recording. On the contrary, a lowest threshold value has to be established in order to distinguish LM from the background EMG noise. ASDA criteria indicate this threshold value on the basis of 25% of the EMG amplitude during the prerecording calibration. Using these criteria, it is unclear how the amplitude of the calibration signal must be measured because it shows evident oscillations, and it is unclear for how long the amplitude of the considered event should remain

over this threshold in order to be classified as a LM. Furthermore, the amplitude parameter is also relevant for the onset and the end of each LM to define precisely its duration and the interval that divides it from the previous and the following ones. In this perspective, the AUC parameter describes the muscle contraction power dissipation better than does the amplitude. Studies carried out by means of surface electrodes and concentric needle EMG analysis have demonstrated that the AUC reflects better the number of myofibers (value depending on the size and the number of motor units) activated during a muscle contraction than does the amplitude.[37] A limit in considering AUC is represented by the fact that its measurement requires a digital automatic program and can not be extracted by visual-manual systems. Nevertheless, computer programs can be arranged to measure the AUC when LM events are also detected manually, as in our case.

The number of inter-LM intervals tends to follow a unimodal-type distribution in young normal controls and a bimodal type in patients with RLS. The difference regarding the first peak (intervals shorter than 8-10 seconds) is significant only for its quantitative aspects but shows a similar shape in both populations. LM separated by less than 4 seconds are excluded by the ASDA criteria[1]; on the contrary, in this study, we found that these intervals were also more numerous in patients with RLS than in normal controls. This indicates the need to also include the analysis of movements separated by less than 4 seconds in the study of the motor patterns of patients with RLS. The choice of 0.5 seconds as a minimum interval to separate 2 consecutive LM and to define monolateral versus bilateral LM might have increased the total number of LM detected by the new analysis; on the contrary, with the classic criteria, many movements during sleep are probably ignored. Thus, in terms of leg motor activity during sleep, the classic scoring criteria might underestimate LM; the impact of this different new approach will probably be evident when it is applied to the analysis of the effects of drug treatment on leg activity.

However, the most striking difference between our patients with RLS and normal controls was the presence of a wide peak, in patients, with a maximum located at around 15 to 30 seconds and extending from 10 to 90 seconds; this peak was clearly absent in normal controls. We believe that this peak primarily represents the periodic EMG activity characterizing RLS, and, for this reason, we decided to establish a tool that is not only able to describe a peak in the distribution histogram (that only gives an estimate of the number of intervals with these characteristics), but also capable of analyzing the time relationships between subsequent intervals. This was achieved by the Periodicity Index, which describes the proportion of intervals in the whole sequence included in "periodic" runs. This index is not influenced by the quantitative aspects of PLM and seems to be stable even along the night in the same individual (data not shown).

An important part of this study was devoted to the analysis of the time structure of the LM-interval sequences by mean of a mathematical approach already known and accepted for this scope (Markovian analysis); this served for the validation and confirmation of the results obtained by means of the simple Periodicity Index .

The Periodicity Index also allowed us to describe 3 arbitrary subgroups of RLS patients, based on the different level of "periodicity" of their PLM. Furthermore, it seems to be useful in providing a new qualitative differentiation among patients with PLM, which is not possible by using the pure quantitative PLM index. Obviously, there is a need for verification and follow-up studies to determine its utility in the discrimination of different patient groups who share, in common, PLMs as a phenotype. These potential differences, not detectable by the standard indexes, might prove to be important, not only in a diagnostic perspective, but also for their possible pathophysiologic meaning. Indeed, different shapes of PLM-interval distribution graphs might reflect important differences in the mechanisms generating LM that might not be exclusively under the control of dopaminergic systems and might involve different and more complex pathways. This point is highly speculative and cannot be resolved by this methodologic study, which only has the scope to propose a new way to analyze a phenomenon and to provide new indexes to be tested more extensively in normal controls and patients affected by different PLM-related pathologies.

REFERENCES

1. Recording and scoring leg movements. The Atlas Task Force. Sleep 1993;16:748-59.
2. Hening WA, Allen R, Walters AS, Chokroverty S. Motor functions and dysfunctions of sleep. In: Chokroverty S, editor. Sleep disorders medicine, 2nd ed. Boston: Butterworth-Heinemann; 1999, p. 441-507.
3. Lugaresi E, Tassinari CA, Coccagna G, Ambrosetto C. Clinical and polygraphic characteristics of Restless Legs Syndrome. Rev Neuro (Paris) 1965;113:545-55.
4. Lugaresi E, Cirignotta F, Coccagna G, Montagna P. Nocturnal myoclonus and restless legs syndrome. Adv Neurol 1986;43:295-307.
5. Montplaisir J, Boucher S, Poirier G, Lavigne G, Lapierre O, Lesperance P. Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria. Mov Disord 1997;12:61-5.
6. Lapierre O, Montplaisir J. Polysomnographic features of REM sleep behavior disorder: development of a scoring method. Neurology 1994;44:987-8.
7. Boivin DB, Montplaisir J, Poirier G. The effects of L-dopa on periodic leg movements and sleep organization in narcolepsy. Clin Neuropharmacol 1989;12:339-45.
8. Ancoli-Israel S, Kripke DF, Mason W, Kaplan OJ. Sleep apnea and periodic movements in an aging sample. J Gerontol 1985;40:419-25.
9. Wetter TC, Collado-Seidel V, Pollmächer T, Yassouridis A, Trenkwalder C. Sleep and periodic leg movement patterns in drug-free patients with Parkinson's disease and multiple system atrophy. Sleep 2000;23:361-7.
10. Mosko SS, Dickel MJ, Paul T, LaTour T, Dhillon S, Ghanim A, Sassin JF. Sleep apnea and sleep-related periodic leg movements in community resident seniors. J Am Geriatr Soc 1988;36(6):502-8.
11. Sforza E, Juony C, Ibanez V. Time-dependent variation in cerebral and autonomic activity during periodic leg movements in sleep: implications for arousal mechanisms. Clin Neurophysiol 2002;113:883-91.
12. Sforza E, Jouny C, Ibanez V. Time course of arousal response during periodic leg movements in patients with periodic leg movements and restless legs syndrome. Clin Neurophysiol 2003;114:1116-24.
13. Coleman RM. Periodic movements in sleep (nocturnal myoclonus) and restless legs syndrome. In: Guilleminault C, ed. Sleeping and Waking Disorders: Indications and Techniques. Menlo Park: Addison-Wesley; 1982;265-95.
14. Parrino L, Boselli M, Buccino GP, Spaggiari MC, Di Giovanni G, Terzano MG. The cyclic alternating pattern plays a gate-control on periodic limb movements during non-rapid eye movement sleep. J Clin Neurophysiol 1996;3:314-23.

15. Allen RP, Hening WA, Picchietti D, Trenkwalder C, Walters AS, Montplaisir J. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology: a report from The RLS Diagnosis and Epidemiology workshop at the National Institute of Health. Sleep Med 2003;4:101-19.
16. The International Restless Legs Syndrome Study Group. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med 2003;4:121-32.
17. Carrier J, Frenette S, Montplaisir J, Paquet J, Drapeau C, Morettini J. Effects of periodic leg movements during sleep in middle-aged subjects without sleep complaints. Mov Disord 2005;20:1127-32.
18. Kemp B, Värri A, Rosa AC, Nielsen KD, Gade J. A simple format for exchange of digitized polygraphic recordings. Electroencephalogr Clin Neurophysiol 1992;82:391−3.
19. Rechtschaffen A, Kales A. A manual of standardized terminology, techniques and scoring system of sleep stages of human subjects. Washington Public Health Service, US Government Printing Office; 1968.
20. Ferri R, Zucconi M, Manconi M, et al. Computer-assisted detection of nocturnal leg motor activity in patients with restless legs syndrome and periodic leg movements during sleep. Sleep 2005;28:998-1004.
21. Gastaut H, Broughton, RA. A clinical and polygraphic study of episodic phenomena during sleep. Recent Adv Biol Psychiatry l965;7:197-221.
22. Oswald I. Sudden bodily jerks on falling asleep. Brain 1959;82:92-103.
23. Wolpert EA. Studies in psychophysiology of dreams: An electromyographic study of dreaming. Arch Gen Psychiatry 1960;2:231-41.
24. Coleman RM, Pollak CP, Weitzman ED. Periodic nocturnal myoclonus occurs in a wide variety of sleep-wake disorders. Trans Am Neurol Assoc 1978;103:230-5.
25. Tauber ES, Coleman RM, Weitzman ED. Absence of tonic electromyographic activity during sleep in normal and spastic nonmimetic skeletal muscles in man. Ann Neurol 1977;2:66-8.
26. Coleman RM. Periodic movements in sleep (nocturnal myoclonus) and restless legs syndrome. In: Guilleminault C, ed. Sleeping and waking disorders: indications and techniques. Menlo Park: Addison-Wesley, 1982;265-95.
27. Broughton R. Pathological fragmentary myoclonus, intensified hypnic jerks and hypnagogic foot tremor: three unusual sleep-related movement disorders. In: Koella WP, Obal F, Schulz H, Visser P, eds. Sleep ‘86. New York: Gustav Fischer Verlag, 1988;240-3.
28. Abramson N. Information Theory and Coding. McGraw-Hill, New York, 1963.
29. Karlsson MO, Schoemaker RC, Kemp B, et al. A pharmacodynamic Markov mixed-effect model for the effect of temazepam on sleep. Clin Pharmacol Ther 2000;68:175-88.
30. Kemp B, Kamphuisen HA. Simulation of human hypnograms using a Markov chain model. Sleep 1986;9:405-14.
31. Ktonas PY, Bes FW, Rigoard MT, Wong C, Mallart R, Salzarulo, P. Developmental changes in the clustering pattern of sleep rapid eye movement activity during the first year of life: a Markov-process approach. Electroencephalogr Clin Neurophysiol 1990;75:136-40.
32. Ktonas PY, Paparrigopoulos T, Monoyiou EA, Bergiannaki JD, Soldatos CR. Sleep spindle incidence dynamics: a pilot study based on a Markovian analysis. Sleep 2000;23:419-23.
33. Jansen BH, Cheng WK. Structural EEG analysis: an explorative study. Int J Biomed Comput 1988;23:221-37.
34. Garcia-Borreguero D, Larrosa O, de la Llave Y, Granizo JJ, Allen R. Correlation between rating scales and sleep laboratory measurements in restless legs syndrome. Sleep Med 2004;5:561-5.
35. Michaud M, Poirier G, Lavigne G, Montplaisir J. Restless Legs Syndrome: scoring criteria for leg movements recorded during the suggested immobilization test. Sleep Med 2001;2:317-21.
36. Michaud M, Paquet J, Lavigne G, Desautels A, Montplaisir J. Sleep laboratory diagnosis of restless legs syndrome. Eur Neurol 2002;48:108-13.
37. Sonoo M. New attempts to quantify concentric needle electromyography. Muscle Nerve 2002;11:S98-102.