Experimentally Induced Arousals Do Not Elicit Periodic Leg Motor Activity during Sleep in Normal Subjects

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Abstract

Objective—To evaluate whether eliciting repetitive cortical and autonomic arousals during sleep is able to induce the occurrence of periodic leg movements during sleep (PLMS).

Methods—Fifteen normal subjects underwent one night of uninterrupted and two sequential nights of experimental sleep fragmentation achieved by auditory and mechanical stimuli eliciting frequent EEG arousals. Sleep was polygraphically recorded and subsequently used to determine the frequency of arousals and occurrence of LM activity during the first (baseline) and the second fragmentation night. Also, heart rate variability parameters were obtained to assess the autonomic changes induced by the stimulation.

Results—Sleep fragmentation was associated with an increase in the arousal index, percentage of sleep stage 1, and frequency of stage shifts. In addition, there was a decrease in sleep latency and in percentage of slow-wave sleep. Moreover, a significant increase in heart rate variability and especially of its sympathetic component, was also found. In contrast, parameters of the leg movement activity showed no significant change following experimental sleep fragmentation. The lack of an increase in leg movement activity was also observed in one subject who demonstrated PLMS at baseline.

Conclusions—Experimental sleep fragmentation is not associated with an increase in PLMS in normal young adults.

Keywords

Periodic leg movement during sleep; PLMS; arousal; autonomic activation; sleep fragmentation; heart rate variability

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1. Introduction

Periodic leg movements during sleep (PLMS) are a common polysomnographic phenomenon characterized by repetitive activations of the tibialis anterior muscle that are typically between 0.5 and 10s in duration and occur approximately every 10–30 s [1]. More than 80% of patients with restless legs syndrome (RLS) manifest PLMS, which may also occur in healthy elderly subjects [2] and other chronic conditions [3]. Dopamine-agonists are effective in suppressing PLMS in patients with RLS, particularly in those with a high frequency of events. PLMS are often accompanied by profound changes in heart rate and blood pressure [4–8], comparable to those observed in association with upper airway collapse in obstructive sleep apnea [9]. Because leg movements that co-occur with obstructive apneas and hypopnea are likely to be pathophysiologically distinct, they are not classified as PLMS according to the current criteria [1,10].

The significance of cortical arousals in eliciting PLMS has been a topic of significant controversy. Periodic increase in electro-cortical and sympathetic activity can occur physiologically during sleep even in the absence of overt PLMS [11–13]. In patients with RLS, the use of pharmacological interventions, such as pramipexole, can decrease the frequency of PLMS without a concomitant decrease in the frequency of arousals [14]. Conversely, reducing the number of arousals by agents such as clonazepam is not associated with a decrease of PLMS [15]. Collectively, these findings suggest that inter-relationships of cortical, motor, and autonomic events during sleep are likely complex and require further clarification.

Important insights on the association between arousals and PLMS can be gained by examining the effects of experimentally-induced arousals on PLMS in normal subjects that are free of comorbid conditions. Thus, the primary aim of the current study was to determine if an increase in the frequency of arousals and autonomic events during sleep is associated with an increase in PLMS. It was hypothesized that, in normal subjects, experimentally-induced arousals would not be associated with an increase in PLMS.

2. Methods

2.1 Study Sample

Healthy volunteers were recruited from the general community. To participate, volunteer subject had to be less than 40 years in age, have a body mass index <30 kg/m^2, consume less than two alcoholic or three caffeinated beverages per day, habitually sleep for at least 7 hours/night, have a usual bedtime before midnight and not work at night or on a rotating shift schedule. Additional exclusion criteria included cigarette smoking and use of any prescription medications or non-prescription anti-inflammatory agents. Eligibility for participation also required absence of the following conditions: type 2 diabetes mellitus, angina, myocardial infarction, coronary revascularization, congestive heart failure, stroke, obstructive lung disease, renal or hepatic dysfunction, psychiatric or neurologic disease. After an initial telephone screening, eligible volunteers were required to complete a serologic screen and an overnight polysomnogram to rule out obstructive sleep apnea as previously described [16]. Usual sleep habits were also objectively assessed with a wrist activity monitor that was worn for at least five days including one weekend. A normal polysomnogram, demonstration of habitual bedtime by midnight and an average of at least seven hours of sleep on actigraphy, and normal serum chemistries were required for enrollment. Most subjects had at least a college education with the exception of one subject who had only completed high school.

After enrollment, multiple contacts were made to counsel each subject on maintaining at least seven hours of sleep per night. Ambulatory monitoring of sleep habits was repeated for three nights prior to the baseline evaluation to confirm that habitual sleep patterns remain unchanged. Volunteers were excluded from participating in the study if sleep duration on any one night was less than six hours or the average sleep duration was less than seven hours preceding admission to the clinical research unit (CRU). Female volunteers were scheduled for the study protocol during the follicular phase (day 4–10) of the menstrual cycle. The experiments were entirely conducted at the Johns Hopkins University, School of Medicine, Baltimore, MD, U.S.A. Informed consent was obtained from each volunteer and the study protocol was approved by the local institutional review board.

2.2 Study Protocol

The study protocol entailed that each subject was admitted to the CRU and had one night of uninterrupted (night 1) and two sequential nights of experimental sleep fragmentation (nights 2 and 3). Results obtained from this study protocol on several outcomes have been previously published [17,18].

2.3 Polysomnographic Recordings

The overnight recording included the following montage: EEG (4 channels, including C3, C4, O1, and O2, referred to the contralateral mastoid); bilateral electrooculograms (electrodes placed 1 cm above the right outer cantus and 1 cm below the left outer cantus and referred to the left mastoid), electromyogram (EMG) of the submentalis muscle, ECG (CM4 derivation: anode in position V6 and cathode attached to the manubrium of the sternum), and one EMG channel combining both tibialis anterior muscles. Sleep signals were digitized using the Somnologica software (Embla Systems) and converted to European data format for further analysis.

2.4 Sleep Fragmentation

Continuous polysomnographic monitoring was performed on each of the three nights in the CRU. Lights out and morning wake times for each subject were matched to their usual bedtimes and wake times and kept constant throughout the three study nights. During the day, subjects were ambulatory in the CRU, but were not allowed to sleep. Sleep fragmentation was achieved by auditory and mechanical stimuli in anticipation of habituation that may occur with a single repeated auditory stimulus type. Auditory tones were broadcast through two speakers placed 12 inches from the head of the bed. Mechanical stimuli were administered using a commercially-available mechanical vibrator. Four such devices were placed underneath the mattress. The aim was to elicit EEG micro-arousals (>3 to <15 s), as defined by standard criteria [10], at a frequency of 30 or more events/hour using the following guidelines; following lights out, two minutes of continuous stage 2 sleep (or higher) were observed before applying the first auditory stimulus, a sine-wave auditory tone of 500 ms duration and 57 dB. If an EEG micro-arousal was not elicited, subsequent stimuli were varied by increasing tone volume in 5–10 dB increments up to a maximum of 100 dB, modulating the frequency of the auditory tone, and applying the mechanical stimulus alone or in combination with the auditory tone. Once an arousal was elicited, at least 30 seconds of stimulus free interval of sleep were required before applying a subsequent stimulus. Arousals were elicited irrespective of sleep stage.

2.5 Sleep, Arousal, and Leg Movement Activity Analysis

Sleep stages were scored using standard criteria on 30 second epochs [19]. Arousals were also scored using standard criteria [10] and the arousal index was calculated as the number of arousals/hour of sleep. Leg movements were recorded and scored on the rectified EMG

signal [20] according to WASM-IRLSSG criteria [1] with visual annotation of all automatic events. Outcomes included the total LM index (total number of leg movements per hour of sleep), PLMS index (entire night and in REM and NREM sleep), as well as the total number of leg movements and number of PLMS sequences. The Periodicity index was calculated as the number of intervals belonging to sequences of at least 3 inter-LM intervals 10<i 90s/ total number of inter-LM intervals. This index can vary between 0 (absence of periodicity) to 1 (all intervals with length 10<i 90s)[21]. The Periodicity Index is independent of the absolute number of leg movements recorded and was calculated for all the subjects. Finally, PLMS associated with arousals [1] were tabulated and the PLMS-Arousal index was determined (number/hour of sleep).

2.6 Analysis of Heart Rate Variability during NREM Sleep Stage 2

In order to study the autonomic system changes induced by the stimulation protocol we decided to analyze heart rate variability (HRV) by focusing our attention on NREM sleep stage 2 because of the extensive presence of this stage in all subjects enrolled, which allowed us to find artifact-free epochs lasting 5 minutes during the second NREM sleep cycle of each recording. During all epochs chosen for HRV analysis, subjects rested in supine or lateral positions.

In each 5 minuteepoch, the ECG signal (sampled at 200 Hz) was analyzed for automatic detection of R waves with Hypnolab 1.2 (SWS Soft, Italy) utilizing a simple threshold plus first and second derivative algorithm. R-R intervals from each epoch were calculated and their value was obtained at regular intervals of 1s by linear interpolation of the measured values, for the first 256 s which were utilized for all subsequent analysis steps.

From these intervals, a series of time-domain measures was calculated:

   a.   mean R-R value, SDNN (standard deviation of all R-R intervals),

   b.   RMSSD (the square root of the mean of the sum of the squares of differences between adjacent R-R intervals),

   c.   NN50 (number of pairs of adjacent R-R intervals differing by more than 50 ms in the entire epoch),

   d.   pNN50 (percentage of NN50 among the total R-R intervals).

Further, the interpolated R-R interval tachograms were also processed by means of a Fast-Fourier Transform (FFT) algorithm and the following spectral parameters were obtained:

   a.   VLF (power in very low-frequency range, < 0.04 Hz);

   b.   LF (power in low-frequency range, 0.04–0.15 Hz);

   c.   HF (power in high-frequency range, 0.15 – 0.4 Hz);

   d.   total power (VLF+LF+HF);

   e.   LF% (LF power in normalized units: LF/(total power – VLF) $\times 100$;

   f.   HF% (HF power in normalized units: HF/(total power – VLF) $\times 100$ and

   g.   LF/HF (ratio LF/HF).

2.7 Statistical analysis

Comparisons between the non-fragmented (baseline) and fragmented nights were made with the use of the nonparametric Friedman ANOVA for paired observations using the commercially available Statistica software package (StatSoft, Inc., 2001. STATISTICA data

analysis software system, Version 6. www.statsoft.com) was used. A p <0.05 was used to define statistical significance.

3. Results

The study sample included 15 subjects (12 males and 3 females, mean age 24.6 years, 4.07 S.D.) that completed the study protocol. One subject was excluded from the analysis because of numerous artifacts in the leg EMG signal at baseline and another subject was analysed separately because of the presence of a PLMS at baseline. The remaining 13 subjects were included in current analysis reported herein.

Figure 1 is an example of a polysomnographic recording fragment from a night of sleep fragmentation demonstrating the differences in spontaneous and evoked EEG arousals (sound meter peaks) evoked by auditory stimuli. The increase in heart rate following an auditory stimulus was significantly higher than the increase observed with a spontaneous arousal. Table 1 includes the parameters of sleep architecture obtained from the non-fragmented (baseline) and fragmented nights. With sleep fragmentation, sleep latency decreased on night 3, the number of stage shifts and of awakenings, as well as the percentage of sleep stage 1 increased, and the percentage of slow-wave sleep was reduced. Not surprisingly, the arousal index was higher on the fragmented nights versus the non-fragmented night.

Table 2 reports the heart rate variability parameters obtained during the three experimental nights. Sleep fragmentation was accompanied by an evident increase in heart rate variability (SDNN and total power at the spectral analysis), which was due especially to the increase of parameters related to its sympathetic component (LF and VLF bands, LF/HF ratio). This increase was evident in both fragmentation nights.

The leg movement activity parameters from the non-fragmented (baseline) and fragmented nights are shown in Table 3. None of leg movement activity parameters tabulated were significantly different between the baseline and fragmented nights of sleep. The distributions of leg inter-movement intervals from the baseline and fragmented nights are shown in Figure 2 (top panel). A peak at approximately 4s with a decrease to 10–12s was observed for all nights. The lower panel of Figure 2 displays the distributions of total leg movements across the sleep period. No statistically significant differences were noted between the non-fragmented and non-fragmented nights.

In the one subject with PLMS at baseline, the arousal index was 9.7 events/hr, the PLMS index was 33.7 events/hr and the periodicity index was 0.75. On the fragmented nights, this subject had an arousal index of 11.1 and 14.0 events/hr, PLMS index of 26.7 and 40.7 events/hr, and a periodicity index of 0.68 and 0.66. Figure 3 compares the distributions of leg inter-movement intervals and the total leg movements during sleep on the non-fragmented and fragmented nights. The stability in leg motor activity is evident across nights given the peak of the inter-movement interval distribution in the 10–40s range and a decreasing number of leg movements across the sleep period on all nights.

4. Discussion

The overarching aim of the current study was to evaluate whether increasing the frequency of arousals and the accompanying autonomic activity would be associated with an increase in PLMS in normal subjects. The results reported herein suggest that an increase in arousals induced by auditory or mechanical stimuli is not associated with an increase in PLMS in normal subjects. In the one subject with evidence of PLMS at baseline, sleep fragmentation and an increase in arousals did not significantly alter the frequency of observed PLMS.

Collectively, these data suggest that, at least in normal subjects, cortical arousals and an increase in autonomic events do not alter the occurrence of PLMS.

Although there is often a clustering of cortical arousals, autonomic events, and PLMS, previous work by our group [5,14,15,22] and others [6–8,23] suggests that the inter-relationships between these events are not likely to follow the simple paradigm of any one event leading to another [24]. The lack of a simple one cause event sequence between these events does not exclude the possibility that, when they do occur concurrently, one modifies the biological effects of the other [25–27]. The resulting effect modification may form the basis of adverse consequences of various clinical conditions in which PLMS are abundant such as RLS [28–31] or periodic limb movement disorder [32,33]. Whether such an effect modification occurs will require further research on the independent and interactive effects of cortical arousals, autonomic events, and PLMS.

PLMS in normal individuals are generally observed after the age of 40 years and usually with a PLMS index that is considerably lower than that observed in our subject with PLMS at baseline [2]. It is now increasingly recognized that PLMS are possibly a genetically-determined phenomenon, as recently demonstrated by a large genome-wide associated study in Iceland [34]. PLMS are considered to be an endophenotype of RLS [35] and gene variants associated with PLMS (i.e., *BTBD9* and *Meis1*) confer an increased risk for RLS [34,36]. In fact, asymptomatic PLMS are often a precursor to RLS and more common in relatives of RLS patients [37] and various in ethnic groups with the highest frequencies of RLS/PLMS risk alleles (e.g., North Americans of European vs. African descent) [38]. It is thus possible that the one subject in the current study with PLMS at baseline with a time structure (intervals and night distribution) identical to that of typical PLMS of RLS [21] may be at risk for developing RLS.

5. Limitations

There are several limitations in the current study that merit discussion. First, only one EMG channel which combined two tibialis anterior muscles was used to record and assess the presence of leg movement activity. While such a montage is not standard, it did not hinder our ability to detect and characterize the presence and severity of leg movements. Second, fragmentation of sleep was only undertaken for two nights and thus inferences are limited regarding the impact of other chronic conditions that are associated with recurrent arousals (i.e., sleep apnea) on PLMS. Third, given the small sample size, the results are not generalizable and are prone to a type 2 error. Finally, we have only analyzed the effects of experimentally-induced arousals; thus, we cannot rule out that an increase in natural arousals in an individual (perhaps with aging) could actually lead to an increase in PLMS. These limitations notwithstanding, there are several strengths in the current study. Given that the effects of sleep fragmentation on PLMS were characterized in normal subjects, the effects of arousals on PLMS were assessed in the absence of numerous confounding conditions (e.g., sleep apnea, habitual sleep deprivation, and smoking). Moreover, the relatively high frequency of experimentally evoked arousals allowed for an assessment of whether frequent arousals can increase the propensity for PLMS.

6. Conclusion

In conclusion, the current study suggests that cortical arousals, autonomic activation, and PLMS, which often occur concurrently, are not related in a simple cause-and-effect sequence. Specifically, increasing the number of arousals does not increase the frequency of PLMS in normal young adults indicating that the pathophysiology of PLMS in various conditions is likely to be more complex than just a simple representation of an arousal response.

Acknowledgments

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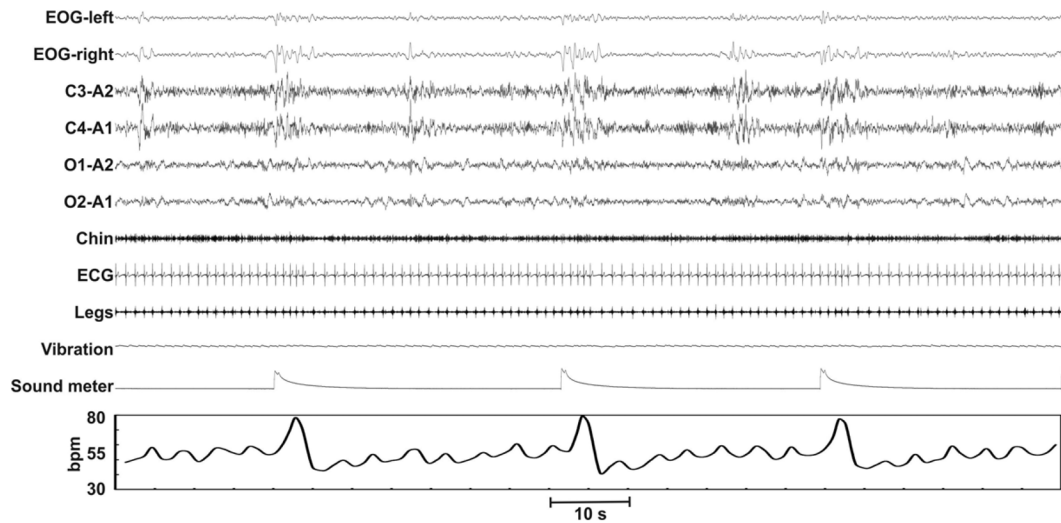
Fig. 1.
Example of a polysomnographic recording during the fragmentation night; heart rate fluctuations are also reported on the bottom. Note the different responses in spontaneous and evoked EEG activations (sound meter peaks) elicited by auditory stimuli and the subsequent rises in heart rate.
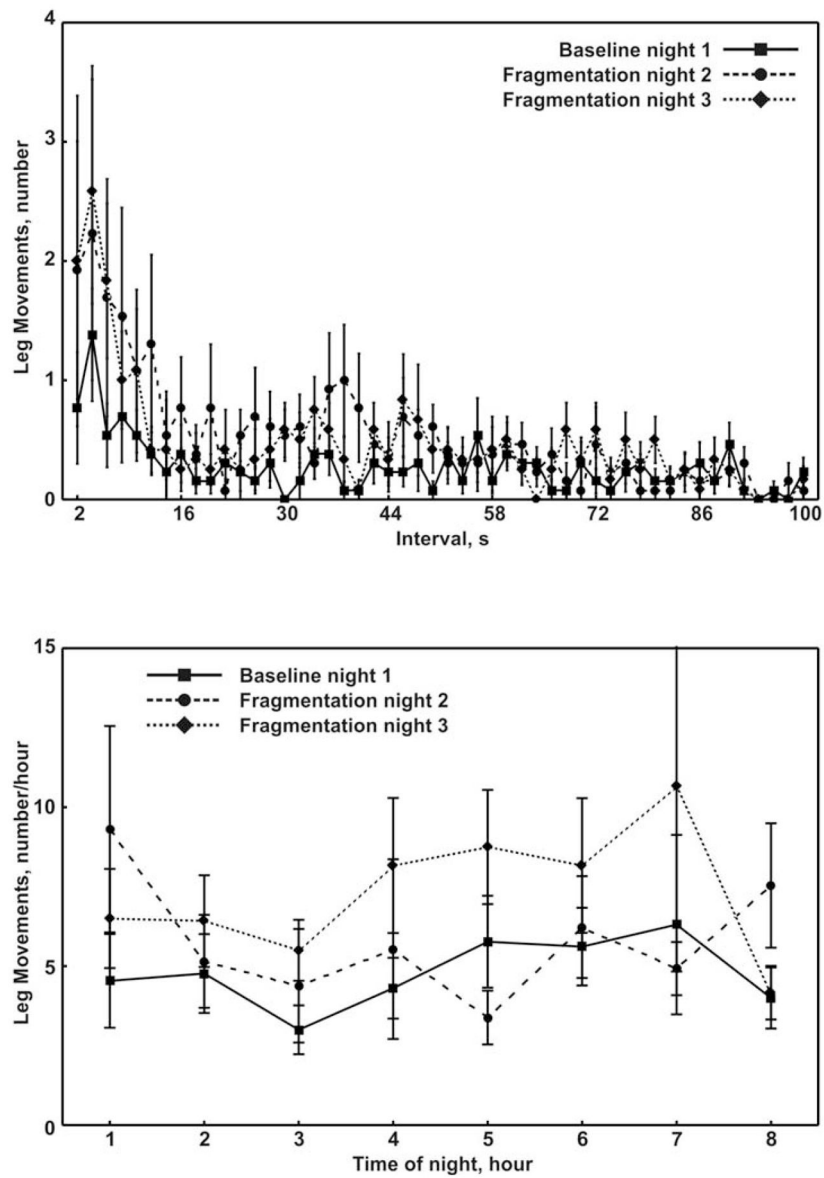
Fig. 2.
Distribution of leg inter-movement intervals (top panel) and total leg movements (bottom panel) during sleep at baseline (non-fragmented sleep, night 1) and during the 2 nights of sleep fragmentation (nights 2 and 3). Values are means and standard error of the means.
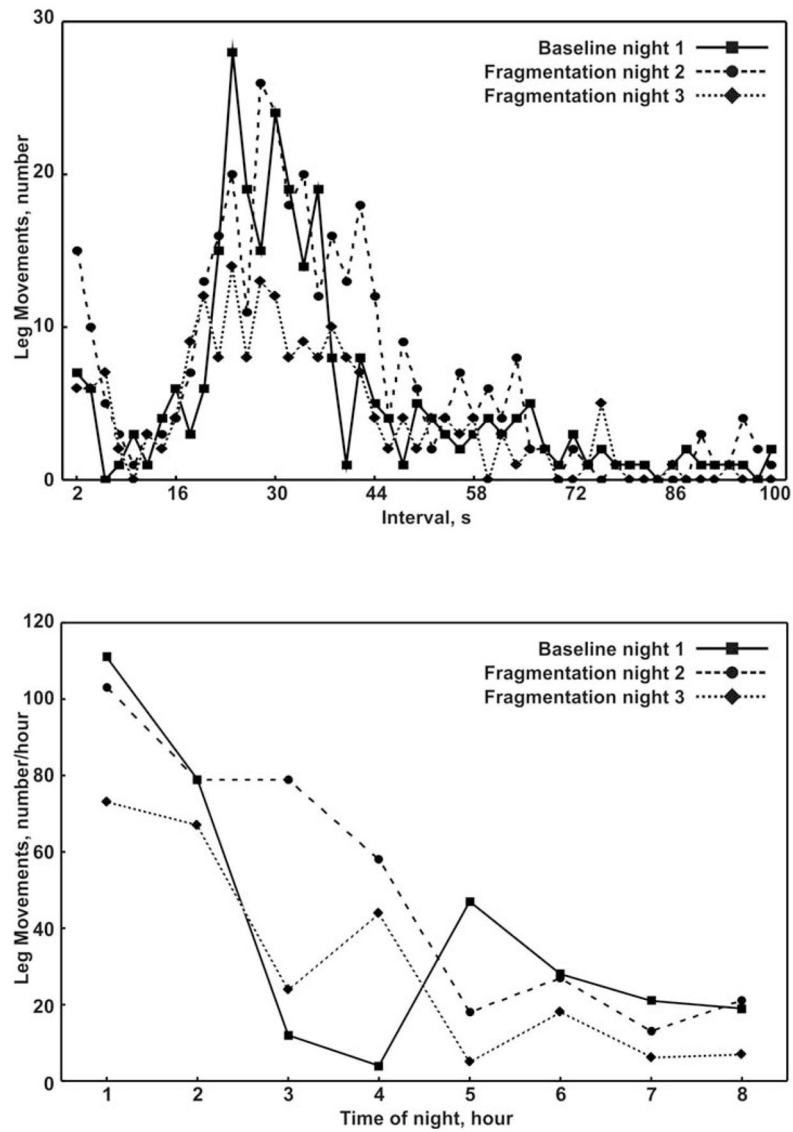
Fig. 3.
Distribution of leg inter-movement intervals (top panel) and total leg movements (bottom panel), at baseline (non-fragmented sleep, night 1) and during the 2 nights of sleep fragmentation (nights 2 and 3), in the single patient with PLMS at baseline.

Table 1

Sleep parameters at baseline (non-fragmented sleep, night 1) and during the 2 nights of sleep fragmentation (nights 2 and 3).

	Baseline night 1		Fragmentation night 2		Fragmentation night 3		Friedman ANOVA p-value
	median	interquartile range	median	interquartile range	median	interquartile range	
TIB, min	480.0	479.5–483.0	480.0	479.0–484.0	479.5	479.0–481.5	NS
SPT, min	474.5	470.5–480.5	476.0	464.0–480.0	477.5	474.5–480.5	NS
TST, min	452.0	437.5–463.5	443.0	428.0–458.5	454.5	438.0–459.0	NS
SL, min	5.5	3.0–10.5	4.5	2.0–16.5	2.5	1.0–16.5	0.02
FRL, min	68.0	63.5–91.0	84.5	78.5–112.0	83.0	73.0–137.0	NS
Stage shifts/hour	11.5	11.3–16.8	21.9	18.8–29.5	22.4	16.1–30.9	0.002
Awakenings/hour	3.4	2.9–4.7	6.6	5.4–8.6	5.9	3.9–7.1	0.014
SE, %	94.3	88.1–96.2	91.7	89.6–95.2	94.5	88.9–95.7	NS
WASO, %	4.0	2.9–7.7	6.0	4.1–8.1	5.1	3.5–9.9	NS
S1, %	3.6	2.8–8.3	16.4	8.4–20.2	10.8	9.3–17.9	0.0002
S2, %	53.3	50.5–58.7	57.2	47.2–59.0	54.8	47.6–58.8	NS
SWS, %	11.9	10.8–16.2	2.3	0.9–3.3	2.1	0.9–7.7	0.001
REM, %	21.8	18.8–23.0	17.5	15.0–21.5	19.8	17.8–21.3	NS
Arousal index, n/hour	14.2	12.0–17.6	36.8	21.9–51.0	30.0	18.8–39.0	0.025

TIB: time in bed, SPT: sleep period time, TST: total sleep time, SL: sleep latency, FRL: first REM latency, SE: sleep efficiency.

Table 2

Heart rate variability at baseline (non-fragmented sleep, night 1) and during the 2 nights of sleep fragmentation (nights 2 and 3).

	Baseline night 1		Fragmentation night 2		Fragmentation night 3		
	median	interquartile range	median	interquartile range	median	interquartile range	Friedman ANOVA p-value
Mean R-R, s	1.04	1.02–1.10	1.10	1.07–1.19	1.14	1.11–1.20	NS
SDNN	0.05	0.04–0.07	0.07	0.08–0.13	0.09	0.07–0.11	0.003
RMSSD	0.05	0.04–0.07	0.07	0.05–0.11	0.06	0.05–0.09	NS
NN50	68.00	40.00–147.00	147.00	50.00–150.00	80.00	58.00–121.00	NS
pNN50	26.67	15.69–57.65	57.65	19.61–58.82	31.37	22.75–47.45	NS
VLF power	0.07	0.06–0.11	0.11	0.12–0.22	0.17	0.13–0.21	0.00025
LF power	0.15	0.12–0.22	0.22	0.25–0.40	0.28	0.24–0.38	0.0001
HF power	0.20	0.11–0.28	0.28	0.17–0.39	0.26	0.18–0.40	NS
Total power	0.39	0.32–0.59	0.59	0.55–1.09	0.70	0.52–0.99	0.003
LF, %	39.91	37.80–44.75	44.75	45.44–58.25	51.44	49.69–57.55	0.00005
HF, %	60.09	55.25–62.20	62.20	41.75–54.56	48.56	42.45–50.31	0.00005
LF/HF	0.66	0.61–0.81	0.81	0.83–1.40	1.06	0.99–1.36	0.00005

SDNN = standard deviation of all R-R intervals; RMSSD = square root of the mean of the sum of the squares of differences between adjacent R-R intervals; NN50 = number of pairs of adjacent R-R intervals differing by more than 50 ms in the entire epoch; pNN50 = NN50%; VLF = power in very low-frequency range (<0.04 Hz); LF = power in low-frequency range (0.04–0.15 Hz); HF = power in high-frequency range (0.15–0.4 Hz); total power = VLF + LF + HF; LF% = LF power in normalized units (LF/[total power–VLF] × 100): HF% = HF power in normalized units (HF/[total power–VLF] × 100): LF/HF = ratio LF/HF.

Table 3

Leg movement activity parameters obtained at baseline (non-fragmented sleep, night 1) and during the 2 nights of sleep fragmentation (nights 2 and 3).

	Baseline night 1		Fragmentation night 2		Fragmentation night 3		
	median	interquartile range	median	interquartile range	median	interquartile range	Friedman ANOVA p-value
NREM sleep							
Total index, n/hour	3.4	2.2–6.3	5.2	3.9–10.5	4.0	2.5–6.1	NS
PLMS index, n/hour	0.0	0.0–0.8	0.4	0.0–1.5	0.0	0.0–1.0	NS
Isolated LMs index, n/hour	3.0	2.2–3.8	5.2	3.8–8.0	4.0	2.5–5.3	NS
REM sleep							
Total index, n/hour	4.6	2.8–7.6	5.8	3.3–9.6	4.4	2.9–8.4	NS
PLMS index, n/hour	0.0	0.0–0.6	0.0	0.0–3.5	0.0	0.0–0.0	NS
Isolated LMs index, n/hour	4.3	2.8–7.6	5.8	3.3–7.6	4.4	3.1–9.1	NS
Total sleep							
Total index, n/hour	3.5	3.2–5.6	6.2	4.3–9.4	4.9	3.6–6.0	NS
PLMS index, n/hour	0.1	0.0–0.7	1.2	0.0–1.3	0.0	0.0–1.0	NS
Isolated LMs index, n/hour	3.2	2.7–4.3	5.7	4.3–8.0	4.0	3.3–5.3	NS
PLMS sequence number	0.0	0.0–1.0	1.0	0.0–2.0	0.0	0.0–2.0	NS
Periodicity index	0.000	0.000–0.118	0.000	0.000–0.092	0.053	0.000–0.077	NS
PLMS/Arousalindex	0.0	0.0–0.4	0.4	0.0–1.1	0.0	0.0–0.4	NS