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Original Article

# 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 leg movement (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.



## 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 10 s 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 interrelationships 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.

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## 2. Methods

### 2.1. Study sample

Healthy volunteers were recruited from the general community. To participate, the 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 h/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 prescrip tion 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 7 h 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 7 h 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 6 h or the average sleep duration was less than 7 h 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, USA. 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/h 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 s 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 s 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 $10 < i \leqslant 90$-s/total number of inter-LM intervals. This index can vary between 0 (absence of periodicity) and 1 (all intervals with length $10 < i \leqslant 90$-s) [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 minutes epoch, 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 1 s 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),