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Original Article

Sleep-dependent motor sequence memory consolidation in individuals with periodic limb movements

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ABSTRACT

Periodic limb movements (PLMs) during sleep increase with age and are associated with striatal neurodegeneration and dopamine deficiency. Limb movements are often associated with disruptions to non-rapid eye movement (NREM) sleep. Motor skill memory consolidation recruits the striatum, and learning-dependent striatal activation is associated with NREM sleep. Therefore, we investigated whether *de novo* individuals who significantly experience elevated levels of PLMs but have not been formally diagnosed with periodic limb movement disorder had learning and sleep-related memory deficits and whether these deficits were related to sleep quality and symptom severity.

In total, 14 adults with significantly elevated PLMs (PLM condition), 15 age-matched controls (CTRL), and 14 age-matched "disturbed" sleep (through induced leg movements) controls (CTRL-ES) participated. The participants were trained (PM) and retested (AM) on procedural motor sequence learning (MSL) and declarative paired associates memory tasks.

Baseline sleep quality was significantly worse in PLM than in CTRL. Despite the continued presence of PLMs in the PLM condition on the experimental night, remarkably, sleep quality improved and arousals decreased, vs. baseline, and did not differ from CTRL. MSL was significantly slower in the PLM condition than in CTRL at training but surprisingly exhibited overnight performance gains, which correlated with reduced arousals. As predicted, CTRL but not CTRL-ES had overnight gains in MSL. Taken together, this suggests that in the PLM condition, sleep quality was normalized following MSL, where they derived the same benefit of sleep to procedural memory consolidation as in CTRL. Sleep did not benefit declarative memory.

Although preliminary, these results suggest that MSL in individuals with PLMs may provide a benefit to sleep, which in turn may benefit memory consolidation.

1. Introduction

Periodic limb movements (PLMs) affect 45% of adults over the age of 65 [1], characterized by stereotyped and repetitive movements of the lower limbs during sleep [2]. These muscle twitches are most frequently experienced in the lower limbs during non-rapid eye movement (NREM) sleep [3]. Overnight polysomnography (PSG) reveals that PLMs are associated with frequent arousals, increased sleep stage shifts [4–6], lowered sleep efficiency (SE) [7], longer sleep onset latency, and shorter sleep duration [8]. Despite a wealth of research characterizing sleep disruption in association with PLMs, the effect of PLMs on the functioning that good quality sleep supports, such as learning and memory consolidation, are not known. The current study aims to take the first steps to lay the groundwork to investigate memory and sleep-dependent memory consolidation deficits in individuals

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who experience clinically significant levels PLMs, but who have not yet sought treatment and are undiagnosed.

The striatum is one of the principle brain areas associated with the occurrence of PLMs. At a neuropharmacological level, degeneration of the striatal dopamine system is believed to contribute to these symptoms. Individuals who experience PLMs have reduced dopamine D2 receptor occupancy in the central nervous system [6] and lower binding to central D2 receptors [9]. D2 receptor activation decreases with age [10], coinciding with an increased prevalence of PLMs in the elderly [11–13]. In addition, limb movements are associated with reduced dopaminergic activity in the striatum, the extent of which is associated with PLM severity [6,14,15]. Thus, it is probable that neurodegeneration of the striatal dopaminergic system would have a negative impact on sleep-dependent memory consolidation, which is also dependent on the striatal system.

Sleep disruption's association with and the underlying pathophysiology of PLMs suggest that NREM sleep-dependent learning and memory consolidation, particularly for motor sequence learning (MSL), would be negatively affected in individuals with a significantly elevated number of PLMs. However, to our knowledge, there are no studies to date that investigate cognitive deficits in individuals who experience PLMs. A large body of evidence exists suggesting that NREM sleep supports the consolidation of procedural motor skill memory consolidation [16–21], which is associated with sleep-dependent striatal activation [22–30]. Moreover, several studies have shown that simple motor skill learning results in robust changes in electrophysiological neural oscillations, which characterize NREM sleep, such as increased sleep spindle activity (e.g., frequency, amplitude, and density) during Stage 2 (NREM2) sleep [16,17,31–35], which is correlated with offline improvements in performance [33,36,37]. More recently, the neural correlates of this phenomena have been identified using functional magnetic resonance imaging, whereby increased blood oxygen level-dependent activity in the striatum, from training to retest, was found to be associated with both sleep spindles and performance improvements [38]; moreover, reactivation of the memory trace occurs time-locked to the incidence of sleep spindles [39]. This suggests that procedural motor skill memory consolidation is dependent on NREM2 sleep, is associated with the characteristic features of NREM sleep such as spindles, and is related to an enhancement of the striatum through the reactivation of the memory trace formed during learning [39]. Declarative sleep-dependent memory consolidation is also related to NREM2 and sleep spindles specifically, but post-learning reactivation involves the hippocampus instead [40] and thus might not be affected in cases with elevated PLMs.

Taken together, these studies suggest that individuals who experience significantly elevated levels of PLMs not only have disrupted sleep associated with the limb movements but also have underlying striatal dopamine deficits, which may collectively have a negative effect on the normal consolidation of procedural motor skills. Consequently, people with PLMs may experience memory deficits that are specific to motor skills whose consolidation is enhanced by sleep. The purpose of the current study was to examine the relationship between significantly elevated levels of PLMs, learning, and memory consolidation.

Given that PLMs are most prominent during NREM2 sleep [3] and have been associated with a reduction in sleep quality [4–8], it remains to be investigated whether consolidation for memory tasks that are dependent on NREM2 sleep (e.g., MSL) is impaired in cases with significantly elevated PLMs. Moreover, it is not clear whether any related impairment in memory consolidation would be due to disrupted sleep *per se*, or independent of this type of sleep disruption (i.e., periodic and brief arousals), or related to other iatrogenic factors associated with PLMs, such as neurodegeneration of brain regions that support motor memory consolidation, e.g., the striatum.

In this study, we take the first steps to investigate the effect of PLMs on motor skill memory consolidation in a sample of *de novo* individuals who experience significantly elevated levels of PLMs but have not been formally diagnosed with periodic limb movement disorder (PLMD) compared to healthy, aged-matched controls. Tests were performed under either normal sleep conditions or by disrupting the sleep of controls by inducing leg movements using mild electrical stimulation of the muscles on the experimental night to mimic PLMs and the associated sleep disruption (but without the underlying neuropathology). Furthermore, to probe whether memory deficits were specific to striatal-dependent motor memory consolidation, we also employed a hippocampal-dependent declarative paired associates (PAs) memory task [41–44], for which consolidation also benefits from NREM2 sleep.

We hypothesized that (**1**) people who experience significantly elevated levels of PLMs will demonstrate sleep-dependent motor skill memory consolidation deficits in comparison to both normal, undisturbed sleep and disturbed sleep conditions in healthy controls and (**2**) only the undisturbed sleep control condition will exhibit motor sequence performance gains (vs. the PLM and disturbed sleep conditions) and normal PA performance gains between the training and retest sessions. This study aims to elucidate whether cognitive deficits in memory that is dependent on brain structures that are associated with increased incidence of PLMs occur in individuals who suffer from significantly elevated levels of limb movements and whether these putative deficits are associated with disturbed sleep that is associated with PLMs.

2. Methods

2.1. Participants

All participants were in good health; had a normal BMI (<30); were right-handed, non-smokers, who did not consume excessive caffeine (<2/day) or alcohol (<5/week); were non-shift workers; had regular sleep between the hours of 10 PM and 9 AM; were free from any medications known to affect sleep; had with no history of chronic pain, seizures, head injury, depression, or anxiety; and had normal mobility of the hands and fingers. Professional typists or trained musicians were also excluded. The first night of PSG recording served as an acclimatization and sleep disorder screening night (see Fig. 1 for the experimental protocol). Electroencephalographic (EEG) activity was recorded from Fz, Cz, Pz, and Oz scalp locations, respiration was measured using thorax and abdomen respiratory effort belts, electrocardiographic activity was recorded from electrodes placed just below the left and right clavicles, leg

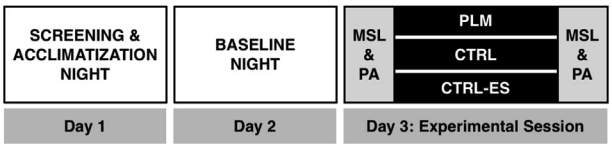

Fig. 1. Experimental protocol. Participants first underwent an overnight screening and acclimatization night to screen for signs of sleep disorders or poor quality sleep, to categorize participants into the PLM or control conditions, and to ensure that a representative baseline recording could be obtained for the following night. The PLM group then underwent motor sequence learning and paired associates training in the evening (PM), followed by overnight PSG, and subsequent retesting the following morning (AM). The control (CTRL) and CTRL-ES groups underwent the same procedure. In the CTRL-ES condition, sleep was disrupted experimentally by inducing leg movements to mimic PLMs. Abbreviations: motor sequence learning (MSL) task; Paired Associates (PA) task; electrical muscle stimulation during sleep to induce periodic limb movements (PLMs).

muscle electromyogram (EMG) tone was recorded from two electrodes placed on the anterior tibialis muscle, and blood oxygen saturation was recorded from an infrared finger probe sensor on the left index finger. The screening night recording was manually scored for sleep staging, arousals, cardiac arrhythmias, respiratory events, periodic limb movements, and any evidence of restless legs syndrome (RLS) and for RLS through the telephone screening interview and related questionnaires (e.g., Sleep Disorders Questionnaire, Pittsburgh Sleep Quality Index) or any other abnormal behaviors that may indicate RLS during sleep. All scoring was completed according to the clinical guidelines established by the American Academy of Sleep Medicine [3] by a single registered polysomnographic technologist (RPSGT) with over 15 years of experience. Participants experiencing >10 respiratory events per hour *(indicating signs of sleep apnea)*, behavioral evidence of RLS, or any other unusual behaviors during sleep were excluded from the study. Control participants, but not PLM participants, with poor sleep quality (SE < 75%) were also excluded from the study. From the results of the acclimatization and screening night, the participants were divided into either the control (<15 leg movements per hour of sleep or no evidence of PLMs) or the PLM (participants exhibiting >15 leg movements per hour of sleep [3]) conditions. Leg movements were scored according to standard clinical criteria [3,45] if they were between 0.5 and 10 s in duration with a minimum amplitude increase of 8 μV in leg EMG voltage (compared to resting leg EMG). The leg movements must also have occurred in a series of at least four movements in the course of 90 s with a minimum interval of 5 s between them. An arousal was scored with a leg movement if there was a 3-s (minimum) burst of alpha activity following the leg movement. However, it should be noted that these research participants were neither seen by a physician nor formally diagnosed as a results of the screening procedure, and thus, we can only assign participants to the experimental conditions depending on the available evidence described here and cannot describe these individuals as having PLMD nor rule out comorbid RLS with absolution. During this visit, participants were also screened for signs of sleep apnea or psychiatric sleep disorders using the Sleep Disorders Questionnaire [46], excluded if they scored above ten on either the Beck Depression [47] and Anxiety Inventories [48], and excluded if they were found to be left-handed, determined by the Edinburgh Handedness Inventory [49]. Finally, all participants completed the Mini Mental State exam [50], and those who scored below 24 were excluded to rule out possible cognitive impairment or signs of dementia. The participants were also asked to wear an "Actiwatch" (Philips-Respironics, Inc., Andover, MA; a wrist-worn accelerometer to measure sleep–wake-related limb movements) and to complete a log of their daily activities and sleep habits to verify that they maintained a regular sleep schedule (bed-time of 10 PM–12 AM and rise-time of 7 AM–9 AM) for 3 days preceding the baseline and experimental nights, and throughout the duration of their participation in the study. Participants were excluded from further participation in the study if the results of their actigraphy or sleep diary indicated non-compliance with maintaining a regular sleep schedule.

Nineteen participants were excluded from the study: eight participants for non-specific poor sleep quality with no evidence of PLMs (SE < 75%), five participants who experienced respiratory events (>10 events/h) on the screening and acclimatization night, and another six participants for either not complying with the experimental protocol or who voluntarily dropped out of the study following the acclimatization/screening night. No participants were found to exhibit evidence of RLS on the basis of telephone interview, questionnaire data, or from the acclimatization and screening night. The final sample consisted of N = 14 adults in the PLM (9 females; M = 50.9, SD = 7.4), N = 15 adults in the undisturbed sleep (CTRL; 13 females; M = 42.2, SD = 13.0), and N = 14 adults in the disturbed sleep (CTRL-ES; 12 females; M = 41.2, SD = 12.9) conditions.

2.2. Ethics statement

All participants were given a letter of information, provided written informed consent prior to participation, and were financially compensated for their participation. This study was approved by the Western University Health Science Research Ethics Board.

2.3. Polysomnographic recording and analysis

Embla Titanium (Natus, Pleasanton, CA, USA) 24 channel EEG systems were used to perform in-laboratory PSG recordings. EEG was recorded at a sampling rate of 512 Hz, with a high pass filter of 0.1 Hz and low pass filter of 220 Hz. EEG (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, and Oz) and electrooculogram (placed on the outer canthus of the eyes) referential recordings (reference Fpz) were re-referenced offline to the averaged mastoid derivations (M1 and M2) and placed according to the international 10–20 electrode placement system [3]. A submental EMG channel was recorded as a bipolar derivation. Leg movements were recorded with the use of two bipolar channels placed on the anterior tibialis muscles of each leg. Manual sleep stage scoring, according to standard criteria [3], was completed by a single scorer using RemLogic analysis software (Natus, San Carlos, CA, USA). The sleep variables of interest obtained from the PSG recording nights included the total sleep time (TST); SE; number of awakenings (NA); wake after sleep onset (WASO); and percentage of time spent in Stage 1 (NREM1) sleep, NREM2, slow wave sleep (SWS), and rapid eye movement (REM) sleep, in addition to leg movements with arousals and without arousals. TST was calculated as the total time spent asleep between "lights off" and "lights on". SE was calculated as a percentage of the total time spent in bed actually sleeping between "lights off" and "lights on" divided by the total time in bed. NA was defined as any 30-s epoch, following sleep onset, that was scored as wake due to either (1) occipital EEG alpha activity occurring for more than 50% of the epoch or (2) a body movement and alpha activity occurring for part of the epoch (even <50% of the epoch) [3]. WASO was defined as epochs following sleep onset that were scored as wake. Sleep stage percentages were calculated as the percentage of time between "light off" and "lights on" scored as NREM1, NREM2, SWS, and REM divided by TST. Leg movements were scored in the same way as the screening/acclimatization night, according to standard clinical criteria, described above [3,45].

2.4. Electrical muscle stimulation

Arousals from sleep experimentally induced by auditory or mechanical stimulation (e.g., vibration) in healthy individuals do not elicit PLMs [51]. Thus, here we simulated PLMs in healthy controls (CTRL-ES condition) with the use of transcutaneous electrical muscle stimulation in individuals who do not naturally experience them at clinically significant levels (e.g., <15 PLMs/h [3]). The low-voltage electrical current stimulation was delivered using a GRASS SD9 (Natus, Pleasanton, CA, USA) stimulator. Two electrodes were applied with adhesive tape onto the participant's right leg directly over the tibialis muscle (about 3–4 inches apart). To induce a brief muscle contraction, the stimulator was set as follows to induce mild muscle contractions of the leg: frequency 0.1 PPS, delay 0.1 ms, and duration 6 ms. The final voltage of the electrical stimulation delivered during sleep was individually determined by raising the voltage gradually until stimulations produced a visible leg muscle twitch, mimicking a naturally occurring limb movement. Feedback from the participant was used

to avoid causing pain or discomfort. Because the number of PLMs differs depending on the time of night, the frequency of the stimulations was varied systematically by NREM cycle [52] and according to age norms [53]. In addition, the frequency and number of stimulations were varied to simulate three PLM severities: severe, moderate, and mild (Table 1). Electrical stimulation was only administered during NREM2 and SWS, when PLMs are most likely to occur [52,54–56]. The administration of electrical stimulation during NREM1 was avoided as it would likely wake participants and prevent them from progressing to the next stage of sleep. The muscle stimulation protocol was terminated after the fourth sleep cycle was complete.

2.5. Objective vigilance

To assess objective vigilance and motor performance, the Psychomotor Vigilance Task (PVT) was administered before each behavioral testing session. The PVT [57] is a computerized reaction time task where participants are required to respond to a visual cue presented at a random inter-stimulus interval as quickly as possible. The PVT testing session included 100 trials (taking approximately 10 min in total duration), where reaction times were measured for each trial.

2.6. Behavioral tasks

2.6.1. Motor sequence learning task

The MSL task was adapted from the finger-tapping task [58]. A numeric keypad was used with four buttons in an ergonomic configuration for the left hand. The task was subdivided into three stages: “verification”, “instruction”, and “training”. First, during the “verification” phase, participants were instructed to execute the sequence 1-2-3-4 only once (where 1 corresponds to the index finger and 4 correspond to the little finger) slowly and accurately to ensure that the equipment and software was operating normally and that the participants were using the keypad as instructed. Next, during the “instruction” phase, participants were instructed to execute a 5-item sequence (e.g., “4-1-2-3-4”) slowly and accurately until the sequence was reproduced three times in a row without making any errors. This procedure was intended to verify that the participants had explicitly learned the sequence and were able to perform the task. During the “training” phase, participants were instructed to execute the sequence learned in the “instruction” phase as quickly and accurately as possible. The training session consisted of 12 blocks of practice (indicated by a green cross in the middle of a black screen) and 12 rest periods between the practice blocks (indicated by a red cross in the middle of a black screen). Each block comprised 60 key presses, and each rest period lasted for 20 s. The participants were instructed to start again at the beginning of the 5-item sequence in the event of an error. The morning retest session was identical to the training session, with the exception that it included only four blocks (60 key presses each) and four periods of rest (20 s each). Three equivalent sequences were randomly assigned to the participants: “4-1-3-2-4,” “2-3-1-4-2,” and “3-4-2-1-3” [30,58]. The measurement of performance on the MSL task was the average time, per block, between each key press for correct sequences.

Table 1
For the disturbed sleep (CTRL-ES) condition, the electrical muscle stimulation (number/h) within each PLM severity condition was varied according to sleep cycle. Participants were randomly assigned to one of three PLM severity conditions (severe, moderate, and mild) to mimic the distribution of severity in individuals in the PLM condition.

	Sleep cycle 1	Sleep cycle 2	Sleep cycle 3	Sleep cycle 4	# of Participants in each condition
Severe	80	60	40	30	4
Moderate	60	40	30	30	5
Mild	40	30	10	10	5

2.6.2. Paired associates task

Participants were randomly presented one of three lists of 40 unrelated word pairs adapted from Payne et al. [59] and Fogel et al. [60]. The participants were instructed to memorize the word pairs by visually relating the words to one another through mental imagery. Each word pair was presented for 5 s, followed by a 5-s rest period. The order of the word pairs was randomized to avoid any order effects. Following this, the participants were presented with one word from the pair (cue) and asked to type in the corresponding word (target). Feedback was provided such that if the target word was incorrect, the participant was presented with the correct pair, allowing for ongoing relearning. A learning criterion of 60% correct answers was determined, and if participants failed to achieve this criterion, the list was randomized and presented again. The training was terminated when 60% learning criterion was achieved. In the morning, participants were tested on the same list of words (randomized once again) with no feedback provided. The performance gains on the PA task were calculated as the change in the number of words correctly recalled from training to retest, e.g., % correct at retest − % correct at training [59].

2.7. Procedure

See Fig. 1 for an illustration of the experimental protocol. All participants were initially screened to verify that they met inclusion criteria (see “Participants” section for details). The initial screening and acclimatization night were used to assign participants to either the PLM (individuals who experienced significantly elevated levels of PLMs, e.g., >15 PLMs/h [3]) or the control (those with <15 PLMs/h or no PLMs) conditions. For the baseline night, all participants returned to the sleep laboratory 4–7 days following the screening night for an overnight recording of a normal night of sleep. Following the baseline night, participants returned to the sleep laboratory for the experimental night. On the experimental night, participants in the PLM and undisturbed sleep (CTRL) conditions were tested in the evening (8 PM) on the PA and MSL tasks, where the order of task administration was counterbalanced across participants. Upon completion of the tasks, participants were allowed to sleep between the hours of 11 PM and 7 AM at which time their sleep was recorded through online PSG, video, and audio recordings. At least 30 min after awakening (to allow sufficient time for sleep inertia to dissipate) [61], participants were retested on the PA and MSL tasks. In addition, prior to and following each behavioral testing session, participants performed the PVT to measure their objective vigilance. The experimental night for the disturbed sleep (CTRL-ES) condition followed the exact same procedure as previously described, except that during the experimental night, their post-training sleep was disturbed by experimentally induced leg movements (see “Electrical Muscle Stimulation” section for details). The participants' sleep schedules and all testing and assessments were carefully controlled for time of day.

3. Results

3.1. Motor sequence learning task

3.1.1. Training session (fast learning phase: blocks 1–8)

A mixed-design 3 (PLM, CTRL, and CTRL-ES) × 8 (training blocks) ANOVA was used to test for changes in performance over the course of the first 8 blocks of the training session between the

PLM, undisturbed sleep controls (CTRL), and disturbed sleep controls using electrical muscle stimulation (CTRL-ES) conditions (Fig. 2A). The results revealed a significant main effect of condition ($F(2,42) = 12.22$, $p < 00.0001$, $h^2 = 0.63$), main effect of block ($F(7, 280) = 74.92$, $p < 00.0001$, $h^2 = 1.92$), and condition by block interaction ($F(14, 280) = 3.92$, $p < 00.0001$, $h^2 = 0.20$). Post-hoc analyses revealed that participants in the PLM condition performed slower overall than those in the undisturbed sleep (CTRL) and disturbed sleep (CTRL-ES) conditions (all $p < 0.01$, all $d > 1.13$); however, participants in the CTRL condition did not differ from those in the CTRL-ES condition on blocks 1 to 8 of the training session. This suggests that all conditions improved similarly across blocks with practice, but the performance of the PLM group was slower overall.

3.1.2. Training session (slow learning phase: blocks 9–12)

To test whether performance had become stable and asymptotic by the end of the training session, a similar mixed-design 3 (PLM, CTRL, and CTRL-ES) × 4 (training blocks) ANOVA was used to compare performance between the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions over the last four blocks of training (Fig. 2A). The results revealed no significant difference in performance across blocks ($F(3, 120) = 2.01$, $p = 0.12$, $h^2 = 0.05$). However, there was a statistically significant main effect of condition ($F(2, 42) = 10.69$, $p < 00.0001$, $h^2 = 0.55$) but no significant (practice block × condition) interaction ($F(6, 120) = 1.18$, $p = 0.32$, $h^2 = 0.06$). These results suggest that performance in all conditions was asymptotic on the last four training blocks, and post hoc tests (all $p < 0.01$, all $d > 1.18$) revealed that the PLM condition continued to perform slower overall (vs. the control conditions) at the end of the training session.

3.1.3. Between-session offline gains in performance

A one-way ANOVA investigated whether there were any differences in % change in MSL performance (i.e., offline gains) between the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions. The results revealed a statistically significant difference between conditions for % change in performance ($F(2,42) = 5.55$, $p = 0.007$, $h^2 = 0.28$; Fig. 2B). Post hoc comparisons indicated significant % change in MSL performance, surprisingly, for the PLM condition ($t(13) = 3.37$, $p = 0.005$, $d = 0.90$, M = 9.13, SD = 10.14) and as predicted for the CTRL condition ($t(14) = 2.34$, $p = 0.035$, $d = 0.60$, M = 4.64, SD = 7.67) but not the CTRL-ES condition ($t(13) = -0.91$, $p = 0.38$, $d = 0.24$, M = −2.37, SD = 9.75). These findings demonstrate that although performance gains on the MSL task were seen in both the PLM and the CTRL condition, performance gains were attenuated with disrupted sleep from induced leg movements in CTRL-ES. This suggests that in healthy adults free from sleep disorders, good quality sleep is necessary for optimal offline memory consolidation to occur.

3.2. Paired associates task

A similar analysis strategy was employed to explore gains in performance for the paired associates task. A one-way ANOVA investigated whether there were any differences in % of recalled words between the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions. The results revealed no statistically significant difference between conditions for % of recalled words ($F(2,42) = 0.38$, $p = 0.69$, $h^2 = 0.02$; Table 2). This suggests that sleep did not preferentially benefit declarative memory consolidation for any of the PLM, undisturbed sleep, or disturbed sleep conditions.

3.3. Psychomotor vigilance task

The PVT was used to assess whether objective vigilance varied between the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions to ascertain whether vigilance was a factor that might explain changes in performance on the memory tasks

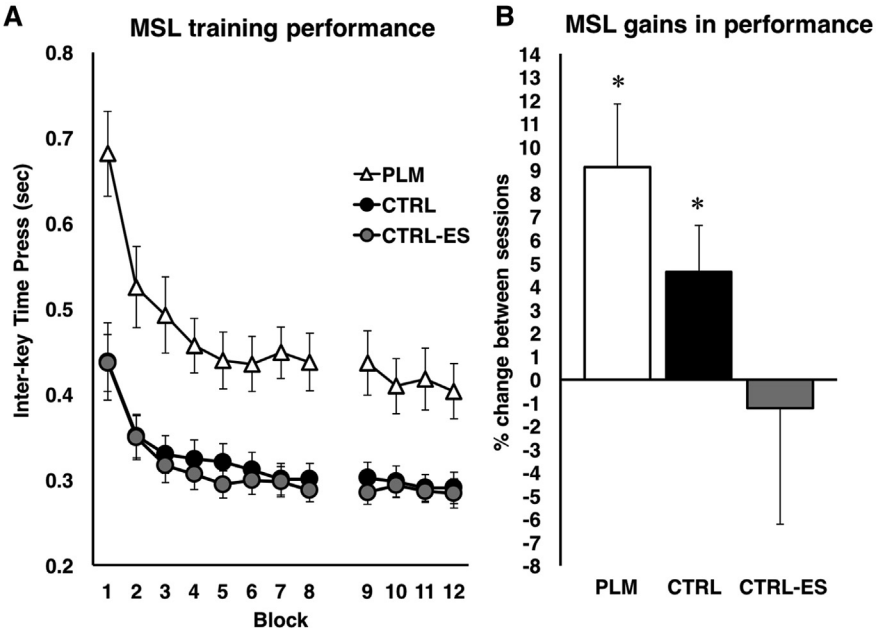

Fig. 2. Behavioral performance. **A**: Performance of the PLM group and the control conditions on the motor sequence learning task during the undisturbed condition (CTRL) and disturbed sleep condition (CTRL-ES) during the 12 blocks of practice (+/−SE). Performance improved over the course of the training during the fast learning phase (blocks 1–8) in all CTRL conditions but was slower overall in the PLM group. Performance was asymptotic in the slow learning phase (blocks 9–12) for all conditions. **B**: Percent change in mean (+/−SE) motor sequence performance between sessions for the PLM, CTRL-sleep, and CTRL-ES conditions. Note: * indicates significant overnight gains in performance from the end of training to the beginning of retest after a night of sleep, at $p < 0.05$.

Table 2
Mean change and SD in % of recalled words of the paired associates task for the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions.

Condition	Performance on the paired associates task (% recalled at training − % recalled at retest)	
	M	*SD*
PLM	2.50	10.05
CTRL	1.00	7.12
CTRL-ES	3.75	8.42

from training to retest sessions or between experimental conditions (Table 3).

A repeated measures 3 (PLM, CTRL, and CTRL-ES) × 2 (training, retest session) ANOVA was used to establish whether the participants from all conditions differed in terms of vigilance before the training and the retest sessions. The results revealed no significant difference in alertness between sessions ($F(1, 42) = 0.029$, $p = 0.866$, $h^2 < 0.001$), no difference in alertness between conditions ($F(2, 42) = 0.004$, $p = 0.996$, $h^2 < 0.001$), and no significant (session × condition) interaction ($F(2, 42) = 0.11$, $p = 0.893$, $h^2 = 0.005$). These results suggest that the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions did not differ on their psychomotor vigilance performance either between sessions or conditions. This suggests that any differences in the MSL task performance may not be attributed to the different levels of psychomotor vigilance performance related to sleep disruption or whether participants suffered in terms of vigilance deficits from PLMs.

3.4. Sleep architecture

3.4.1. Sleep quality and quantity

A repeated measures 2 (baseline and experimental night) × 3 (PLM, CTRL, and CTRL-ES) ANOVA was used to investigate changes in sleep architecture and leg movements (Table 4) from the baseline to the experimental in PLM and control conditions. There was a significant main effect of condition whereby the PLM group had lower TST ($F(2,42) = 9.95$, $p < 0.0001$), reduced SE ($F(2,42) = 6.82$, $p = 0.003$), increased NA($F(2,42) = 4.50$, $p = 0.017$), and increased WASO ($F(2,42) = 6.80$, $p = 0.003$) but no significant interaction effects for percent TST of NREM1, NREM2, SWS NREM, or REM sleep. Thus, sleep architecture did not differ from the baseline to the training night as a function of experimental condition, however, sleep quality was affected. Follow-up t-tests on TST, sleep efficiency, NA, and WASO revealed that the PLM condition differed from the CTRL condition ($t(27) = 3.14$, $p = 0.002$, $t(27) = 2.81$, $p = 0.009$, $t(27) = -2.52$, $p = 0.018$, $t(27) = -2.70$, $p = 0.012$) and the CTRL-ES condition ($t(27) = -3.18$, $p = 0.004$, $t(27) = -2.76$, $p = 0.011$, $t(27) = 2.44$, $p = 0.022$, $t(27) = 2.60$, $p = 0.015$) on the baseline night, but the CTRL and CTRL-ES conditions did not differ from one another (all $p > 0.8$). However, interestingly, follow-up t-tests on the training night surprisingly revealed that the PLM condition differed from the CTRL condition only in terms of TST ($t(27) = 2.42$, $p = 0.023$) and from the CTRL-ES condition only in terms of TST and SE ($t(27) = -2.52$, $p = 0.018$, $t(27) = -2.23$, $p = 0.034$), whereas the CTRL and CTRL-ES conditions did not differ from one another (all $p > 0.5$). This suggests that sleep quality in the PLM condition was normalized following learning on the MSL task.

Table 3
Mean (SD) psychomotor vigilance task (PVT) speed (ms) for the training and retest sessions in the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions.

	Condition		
	PLM	CTRL	CTRL-ES
PVT at training	310.75 (41.23)	312.98 (25.47)	313.47 (19.03)
PVT at retest	314.39 (37.41)	311.61 (36.10)	313.46 (59.57)

3.4.2. Leg movements

A similar approach revealed that there was a significant night by condition interaction whereby the CTRL and PLM conditions differed consistently on both nights, but the CTRL-ES condition did not differ from the PLM condition in leg movements on the experimental night for total number of leg movements ($F(2,42) = 37.02$, $p < 0.0001$), leg movements with arousals ($F(2,42) = 29.37$, $p < 0.0001$), and leg movements without arousals ($F(2,42) = 25.27$, $p < 0.0001$). Follow-up pairwise comparisons revealed that only the CTRL-ES condition showed a significant change for all types of leg movements from the baseline to the training night (all $p < 0.001$). Interestingly, there was a marginally significant reduction in leg movements with arousals in the PLM condition from baseline to the training night ($t(13) = 1.87$, $p = 0.084$), which was statistically significant when controlling for interindividual differences at baseline ($t(13) = 2.2$, $p = 0.046$). There was no change in leg movements in the CTRL condition. This suggests that electrical stimulation in the CTRL-ES condition on the experimental night induced similar leg movements as in the PLM condition, and there was a significant and unexpected reduction in leg movements with arousals following MSL training in the PLM condition, which is in line with the observed improvements in sleep quality.

Table 4
Mean (SD) of the sleep architecture variables for the baseline and experimental nights for the PLM, undisturbed sleep (CTRL), and disturbed sleep (CTRL-ES) conditions.

Sleep characteristic	Condition					
	PLMs		CTRL		CTRL-ES	
	Baseline	Experimental	Baseline	Experimental	Baseline	Experimental
Total sleep time (h)	6.33 (0.71)	6.43 (0.65)	7.17 (0.62)	6.90 (0.35)	7.13 (0.62)	6.95 (0.41)
Sleep efficiency (%)	87.37 (8.77)	90.93 (6.32)	94.39 (3.96)	94.31 (3.68)	94.49 (4.09)	95.16 (3.18)
# of awakenings	21.93 (11.78)	19.43 (10.83)	13.47 (5.40)	13.47 (6.80)	13.43 (5.60)	13.43 (6.80)
Wake after sleep onset (min)	49.02 (38.55)	32.57 (26.19)	19.62 (16.74)	16.62 (14.05)	19.59 (17.37)	17.10 (12.34)
% NREM 1	5.25 (3.78)	4.14 (2.94)	3.06 (2.05)	2.76 (2.49)	3.03 (2.12)	2.94 (1.80)
% NREM 2	54.56 (8.87)	51.47 (9.57)	53.54 (9.25)	50.74 (8.33)	53.05 (9.40)	55.63 (12.47)
% NREM 3	20.77 (5.76)	23.33 (7.43)	21.49 (8.66)	22.99 (6.51)	21.94 (8.80)	20.81 (7.82)
% NREM (total)	80.58 (5.77)	78.94 (6.49)	78.06 (4.12)	76.49 (4.55)	77.99 (4.27)	79.37 (8.26)
% REM	19.42 (5.77)	21.06 (6.49)	21.91 (4.09)	23.51 (4.55)	21.98 (4.24)	20.62 (8.27)
# leg movement (total)	165.50 (87.54)	163.29 (84.08)	2.47 (7.47)	8.07 (14.10)	2.64 (7.72)	169.71 (54.61)
# leg movements with arousals	62.93 (27.62)	54.86 (29.11)	1.00 (2.45)	4.40 (7.55)	1.07 (2.53)	42.21 (20.01)
# leg movements without arousals	102.57 (77.66)	108.43 (66.06)	1.47 (5.14)	3.67 (6.94)	1.57 (5.32)	127.50 (53.23)
Leg movement duration (s)	1.46 (0.37)	1.44 (0.40)	1.23 (0.40)	0.82 (0.67)	1.20 (0.39)	0.84 (0.67)

3.4.3. Questionnaire data

One-way ANOVAs revealed that there was no significant differences in age ($F(2, 42) = 3.05$, $p = 0.06$), Beck's depression scores ($F(2, 42) = 0.01$, $p = 0.99$), Beck's anxiety scores ($F(2, 42) = 0.97$, $p = 0.39$), Mini Mental State Exam ($F(2, 42) = 1.08$, $p = 0.35$), the Stanford Sleepiness Scale on the screening night ($F(2, 42) = 0.21$, $p = 0.82$), on the Sleep Disorders Questionnaire subscales for Sleep Apnea ($F(2, 42) = 1.88$, $p = 0.17$), movement disorders ($F(2, 42) = 0.23$, $p = 0.80$), or psychiatric disorders ($F(2, 42) = 0.51$, $p = 0.60$), on the Epworth Sleepiness Scale ($F(2, 42) = 0.03$, $p = 0.97$), Circadian Rhythm Questionnaire ($F(2, 42) = 0.08$, $p = 0.92$), or the Pittsburgh Sleep Quality Index ($F(2, 42) = 0.11$, $p = 0.90$).

3.5. Relationship between sleep quality and offline gains in MSL performance

Multiple regressions were used to follow-up whether improved sleep quality (e.g., TST, sleep efficiency, NA, and WASO) was related to offline gains in MSL performance when contrasting the PLM vs. CTRL condition and the PLM vs. CTRL-ES condition. These analyses revealed that there was a significant relationship (Fig. 3) between offline gains in performance and increased TST and also between offline gains in performance and reduced NA when contrasting the PLM vs. CTRL condition ($t = -2.37$, $p = 0.023$, $\beta = -0.42$; $t = 3.59$, $p = 0.001$, $\beta = 0.72$, respectively) and between the PLM vs. CTRL-ES condition ($t = -2.54$, $p = 0.019$, $\beta = -0.37$; $t = 4.11$, $p < 0.001$, $\beta = 0.71$, respectively). This suggests that the unexpected offline gains in performance in the PLM condition as compared to the CTRL conditions on the training night was related to the unexpected improvement in sleep quality in terms of increased TST and reduced NA.

4. Discussion

The current study aimed to investigate motor skill learning and memory consolidation in individuals who experience significantly elevated PLMs but have not been formally diagnosed with PLMD compared to healthy, aged-matched controls under either undisturbed sleep conditions or with sleep disrupted as a result of electrically induced leg movements. Results of the current investigation revealed that (1) individuals with PLMs exhibited slower performance overall than controls when learning a procedural motor skills task; (2) these deficits appear to be specific to MSL, *per se*, as no impairment was observed for a declarative paired associates memory task and no differences in psychomotor vigilance were observed; (3) individuals in the disturbed sleep condition had significantly more leg movements than controls, and their motor sequence performance gains were attenuated with disrupted sleep. Contrary to our hypotheses, (4) individuals who experienced PLMs surprisingly showed overnight motor sequence performance gains as compared to normally rested controls, (5) the extent of which was correlated with the restoration of normal sleep quality as compared to controls on the training night.

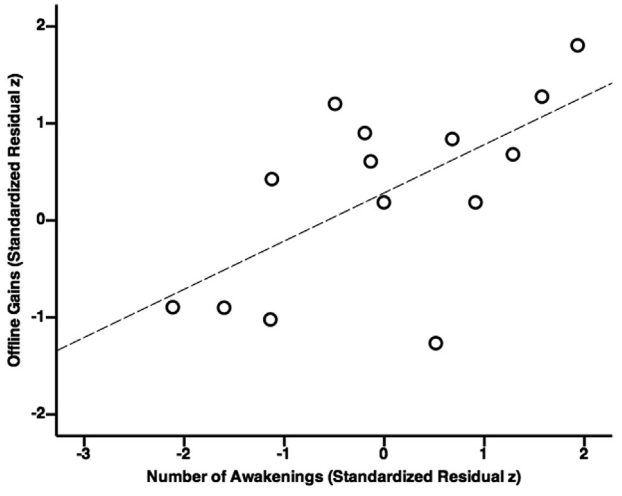

Fig. 3. Relationship between offline gains in MSL and number of awakenings in the PLM condition. The reduction in the number of awakenings in the PLM condition from the baseline to the training night was correlated with the offline gains in performance for the MSL task.

Despite chronically disrupted sleep [4–6,8] and known neurodegeneration in the striatal dopaminergic system [6,9,14,62] in cases with elevated PLMs, to our knowledge, there have been no studies investigating the behavioral consequences of PLMs on sleep-dependent memory consolidation. Here we found, for the first time, that *de novo* individuals with significantly elevated PLMs had overall slower performance than controls during the training session for a simple motor procedural sequence learning task, although they importantly exhibited a normal pattern of learning otherwise. Given that there were no differences in psychomotor reaction time, this difference in MSL performance cannot be easily attributed to increased sleepiness and fatigue due to impoverished sleep quality associated with PLMs or to global impairments in motor execution or performance. Moreover, slower performance in MSL during the training session was specific to motor skills and not a more global memory deficit, given that the PLM group performed normally on the declarative PA task. This suggests that declarative memory may be unaffected in cases with significantly elevated PLMs. Additional support for the notion that this deficit was not due to more global cognitive deficits is supported by the fact that there were no signs of mild cognitive impairment, as determined by the Mini Mental State exam, in individuals with PLMs. Together, these findings suggest that individuals who experience PLMs demonstrated deficits specifically in the speed of performance for procedural sequence learning but not in declarative memory or in general cognitive functioning. While speculative, this pattern of results is consistent with neurodegeneration in the striatum and the dopamine system observed in PLMD [6,9,14,62] and is a potential avenue for future research to investigate the neural correlates of cognitive deficits in individuals with PLMs. There are several potential explanations for why individuals with PLMs may have impaired motor skill learning. First, patients who suffer from both RLS and PLMs have limb movements associated with dysfunctions in striatal brain structures, including the putamen and, to a lesser degree, the caudate [6,14,15]. Given that MSL is dependent on the striatum [25,26,28,30,33,38,63–65], it would not be surprising if individuals who suffer from significantly elevated limb movements were impaired on such tasks. Functional/structural differences in the putamen may underlie this impaired MSL and remains to be investigated. Second, individuals who suffer from PLMs experience reduced sleep quality, possibly resulting from the limb movements themselves during sleep [5,8]. However, this does not result in excessive daytime sleepiness [54,66,67], suggesting that memory performance may not be affected by excessive daytime sleepiness due to possible PLM-related sleep disruption. Consistent with this notion, objective sleepiness in the present study, as assessed by the PVT, did not differ between PLM and control conditions [68]. Thus, our results suggest that slower performance on MSL in undiagnosed *de novo* individuals who experience PLMs may be associated with neurodegeneration in the striatum rather than being attributable to reduced sleep quality affecting daytime performance. However, a major shortcoming of the present study was that we did not directly assess whether

slower performance on MSL was mediated by structural or functional striatal abnormalities in individuals with PLMs and is an important area for future studies to investigate. Additionally, research on structural deficits in these individuals is rather limited. Therefore, this could be an important area for future research, especially considering that the participants in the current study were undiagnosed and only exhibited clinically significant signs of PLMs but were neither aware of their condition nor ever sought or received diagnosis or treatment. Thus, slower performance on MSL may serve as a behavioral early warning sign for the development of PLMD. However, this remains to be explored in individuals who have been formally diagnosed with PLMD.

While sleep disruption associated with PLMs does not necessarily result in excessive daytime sleepiness, it would be expected that these sleep disruptions might interfere with the functions that sleep supports, such as memory consolidation. However, in the present study, paradoxically, the PLM group demonstrated sleep-related offline gains in motor sequence performance. While this may seem counter-intuitive, studies in animal models have suggested that physical exercise stimulates the synthesis of dopamine, leading to motor performance improvements (for review, see Ref. [69]). This is also supported by human studies that showed elevated levels of dopamine following aerobic exercise [70]. In addition, patients with Parkinson's disease who participated in a 3-week exercise program showed significant movement initiation and reaction time improvements in a simple reaction time task [71]. Other studies on patients with Parkinson's disease have shown that goal-based exercises (e.g., Tai Chi, dancing, and boxing) that involve repetitive movements lead to improvements in motor performance [72]. While these studies employed larger muscle groups compared to the current experiment and although speculative, these findings suggest that physical training on the MSL task may have elevated dopamine levels, resulting in PLM condition improvements on the motor sequence task. However, the current study was not explicitly designed from the outset to test this possibility, so this question remains to be directly investigated in future studies. Another possible, albeit speculative explanation for these findings is that MSL practice in the PLM group may have had a beneficial effect on post-learning sleep. Previous research has found that sleep architecture is radically altered and reorganized following intense periods of motor skills learning [16]. Consistent with these results, we found that post-training sleep quality was normalized in the PLM condition from the baseline night to the experimental night when compared to the controls. This improved sleep quality was marked by a reduction in the number of PLMs associated with arousals. This suggests that the unexpected offline gains in performance in the PLM condition as compared to the CTRL conditions on the training night was related to the unexpected improvement in sleep quality in terms of increased TST and reduced NA. At present, the only available treatment for PLMD is the use of dopamine agonist medications [73–79]. However, while purely hypothetical, this study suggests that engaging the striatum prior to sleep through training on novel tasks that rely on the striatum, such as motor sequence learning, may have a beneficial effect on sleep quality by reducing the number of possible PLM-related arousals during subsequent sleep, allowing sleep-related memory consolidation to proceed with less disruption. This hypothesis and novel and unexpected pattern of results, however, remain to be investigated, employing an experimental approach specifically designed to test such a hypothesis. Nonetheless, this could represent a potential avenue for non-pharmacological treatment of PLMD, but this rather contentious possibility remains to be directly investigated.

Consistent with our predictions, the undisturbed sleep control condition demonstrated performance gains on the MSL task, but the performance gains were attenuated in the disturbed sleep control condition. It should be noted that the sample sizes were relatively small (although consistent with the extant literature on sleep and memory in healthy and clinical populations). The samples also predominantly consisted of women, although the ratio of women to men is consistent across the experimental conditions, and thus, there should be no gender bias for the main experimental manipulation. However, the possibility remains that the effects observed here may predominate in women, and this remains to be explored in a larger sample where gender differences can be explored. These results demonstrate that even a very mild amount of sleep disruption in healthy individuals result in a significantly reduced benefit of sleep to motor sequence memory consolidation and may appropriately be a sensitive outcome for the current study's aims to detect the effects of NREM sleep disruption on memory consolidation due to significantly elevated levels of PLMs.

5. Conclusions

At present, while relatively much is known about the prevalence, biological basis, and sleep disturbances related to limb movements, very little is known about associated cognitive and memory deficits, and the present study is an important first step in addressing this important knowledge gap. The current results suggest that individuals who experience significantly elevated levels of PLMs but have not been formally diagnosed with PLMD exhibit improvement with practice on motor skills learning; they also exhibit slower performance overall and do not reach the same level of performance as normally rested, age-matched controls. This slower performance in procedural learning is specific to simple motor procedural memory as declarative memory was not impaired; moreover, there were no signs of mild cognitive impairment or evidence to suggest that performance deficits on the MSL task were due to excessive daytime sleepiness. This suggests that deficits in MSL may be related to the underlying neural deficits known to be associated with PLMs; however, this possibility remains to be explored in future studies. Interestingly, individuals with significantly elevated PLMs showed an offline improvement in performance on the MSL task, similar to the healthy control group. Following motor sequence learning, sleep quality was improved in the PLM condition as indicated by a reduction of PLM-associated arousals, suggesting that MSL may have had a therapeutic effect on sleep quality. However, this possibility remains contentious and requires further experimental confirmation.

In summary, this study suggests that PLMs may not simply have negative consequences on sleep quality; rather, one of the associated features of PLMs may be cognitive. Finally, this research may lead to promising avenues for identifying early warning signs of neurodegeneration and novel non-pharmacological management of PLMD; however, this possibility remains to be investigated.

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Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2017.09.005.

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