Occurrence of limb movement during sleep in rats with spinal cord injury

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Abstract

Several studies have shown the occurrence of Periodic Leg Movement (PLM) in spinal cord injury patients. The aim of this study was to identify the occurrence of limb movements during sleep in spinal cord injury rats and the possible involvement of the spinal cord in causing these movements. The animals were allocated to spinal cord injury (SCI) and SHAM groups. The two groups were submitted to surgery and electrodes inserted to analyze sleep patterns (electroencephalogram—ECoG) and muscular activity patterns (electromyogram—EMG). After baseline sleep recording (24 h), the spinal cord injury surgery (level T9) was performed on the SCI group rats and sleep was recorded for seven consecutive days. After spinal cord injury, 10 of the 11 rats began to present limb movements during sleep, while the SHAM group showed no limb movements during the 8-day sleep-recording period. In relation to sleep efficiency, the SCI group presented alterations during the first few days after spinal cord injury but returned to normal values at the end of the 7-day experimental period. The data suggest that spinal cord injury rats may be used as models to study PLM in paraplegic patients, and that these movements may be generated in the spinal cord itself, without the involvement of the cortical structures.

1. Introduction

Periodic Limb Movements (PLM) are stereotyped movements of lower limbs, that appear specially during light sleep stages 1 and 2 and usually take place at 5–90 s intervals [1]. PLM affects 6% of the population and may occur associated with the restless legs syndrome (RLS) [3]. This disturbance may lead to reduced sleep efficiency and quality.

The precise origin and pathophysiological mechanisms for PLM are obscure. However, there are descriptions in the literature of the possible involvement of the spinal cord.

Though reduced cortical inhibition [16] or impairment of cortical–sub cortical motor structures, in particular of motor inhibitory pathways, has been reported in RLS, a cortical origin for PLM is unlikely because back-averaging studies failed to disclose any cortical potentials preceding PLM. However, the occurrence of PLM in patients with spinal cord injury suggests that PLM may be directly generated in the spinal cord [4,5,6,9,20].

Having studied 10 physically disadvantaged subjects (two of them spinal cord injury patients) and observing leg movements during sleep, Yokota et al. [20] suggested that PLM of spinal cord origin may be induced by interruption of a tract distinct from but close to the corticospinal tract. In another study, Lee et al. [9] described abnormal lower limb movements during sleep in three patients with spinal cord injury at the thoracic level and related them to PLM. De Mello et al. [4–6] have described treatment of PLM based on physical exercise and dopaminergic agonist drugs, and a positive correlation between K-complex and PLM.

The findings of Hains et al. [8] demonstrated dynamic plasticity in properties of dorsal horn somatosensory neurons after SCI (rats).

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Research into the origin and development of this sleep disorder has involved animal models with the aim of clarifying certain aspects yet to be explained.

A study involving subcortical lesions to the A11 dopaminergic nuclei [13] and another examining the age effect for this phenomenon in rats [2] found positive results for certain PLM parameters.

In view of the previous reports of PLM in paraplegic individuals, an important step was to observe the incidence of limb movements during sleep in spinal cord injury animals in an attempt to elucidate the key causative factors.

2. Methods

2.1. Subjects

Nineteen male Wistar rats (90 days) were used for the experiment. Rats were kept individually in transparent cages, under controlled 12-h light–dark cycle conditions (lights on at 7:00 h) at a temperature of 23 ± 1 °C, with unrestricted access to water and food. All experimental procedures were submitted to and approved by the Ethics Committee of Universidade Federal de São Paulo.

2.2. Groups

The rats used were divided into two groups:

- SHAM rats (n = 8)—only cutaneous incision around the spine was performed (not extending to more severe injury) and the spinal cord was not affected;
- Spinal cord injury rats—SCI (n = 11)—spinal cord lesion was performed at level T9.

The rats were submitted to the following procedures: electrode insertion surgery, 24-h duration baseline sleep recording, spinal cord injury (level T9) and subsequent sleep recording for seven consecutive days.

The same spinal cord injury surgery was performed on all SCI group rats, but after the histopathological study were registered four different lesions levels in the spinal cord. All the different levels found in surgery were included in the experiment.

2.3. Surgical preparation

The rats were anesthetized with Diazepan and Ketamine (5 and 100 mg/kg body weight, i.p.); they were placed in the stereotaxic apparatus and five pairs of electrodes were inserted. For the eletrocorticography recording (ECoG) with a minimum of theta activity, one pair of screw electrodes were inserted in the skull ipsilaterally in the lateral position: 1 mm posterior to bregma, 3 mm lateral to central suture; and 1 mm anterior to lambda, 4 mm lateral to the central suture. To ensure the best recording of theta activity, two screw electrodes were placed ipsilaterally in a more medial posterior position: 3 mm anterior to bregma, 1 mm lateral to the central suture; and 4 mm anterior to lambda, 1 mm lateral to the central suture [15]. For the electromyography recording (EMG), three pairs of electrodes were inserted in the skeletal musculature (both hind limbs-gastrocnemius and cervical musculature). After electrode insertion surgery, the rats were placed in individual compartments for 10 days recovery and then given 4 days adaptation while connected for polysomnographic recording (PSG).

On day 16 after electrode insertion surgery the rats were submitted to spinal cord injury surgery. The rats were anesthetized with Diazepan and Ketamine (5 and 100 mg/kg of body weight, i.p.) and placed in the stereotaxic apparatus. The spinous process was detected by tactile probing. Laminctomy was performed and the ninth thoracic vertebra (T9) exposed and removed from the spine. The spinal cords of the SCI group rats were sectioned transversally using a surgical blade. Muscles and skin were sutured. In the SHAM group an incision in the skin and muscles at level T9 was made but no spinal cord injury. The entire surgical procedure was conducted with the assistance of an electronic magnifying device.

2.4. ECoG recording

During recording the rats remained in individual compartments with unrestricted access to food and water.

PSG recording was made by a Nihon-Koden model QP 223 polygraph (digital signal acquisition), using five pairs of channels: two ECoG, one EMG for the cervical musculature and two EMG channels for right and left hind limbs.

The animals were submitted to two polysomnographic (PSG) recording periods: baseline PSG recording for 24 h and PSG for seven consecutive days starting 24 h after spinal cord injury surgery. The recording was analyzed for two 12-h periods (12-h light–dark), whereas it is demonstrated in the literature that the rats’ sleep pattern shows 62% sleep efficiency during the light period (7:00–19:00 h) and 33% during the dark period (19:00–7:00 h) [7].

Each 30-s period was classified in accordance with Timo-Iaria et al. [17]. Wakefulness (W) was defined as low amplitude waves with fast ECoG and EMG activation; non-REM Sleep (Non-Rapid Eye Movement) as high-amplitude waves and slow ECoG and EMG activation; paradoxical sleep (PS) was characterized as fast ECoG activation, regular presence of theta hippocampus rhythm and absence of EMG activity. At the end of the analysis, sleep parameters were quantified using the Polysmith Software program®.

The sleep parameters collected were: sleep efficiency (percentage of total sleep time during recording period); latency to sleep (time lag between starting the recording and
the first sleep period), total awake time (TWT-percentage of all periods of wakefulness throughout the recording period), non-REM (Non-REM-percentage of all periods featuring high delta content during the recording period), PS (percentage of all Paradoxical Sleep periods during the recording period), number of awakenings and number of rats moving limbs during non-REM sleep.

2.5. Analysis of limb movements

Analysis of limb movements was made using electrodes inserted in the two hind limbs (observed during PSG), to characterize flexion and extension of the hind limbs taking place during Non-REM sleep, associated with increased EMG amplitude, periodically or as isolated occurrences. Paws movements lasting 3 to 9 s were taken into account. Video analysis was made at the same time as PSG recording by observing the behavior of the rats.

2.6. Histopathological study

The rats were sacrificed using chloral hydrate (lethal dose) and the spinal cord removed and placed in 10% buffered formalin. Then samples from the cervical, thoracic, lumbar and sacral levels were kept in paraffin, as per routine optical microscope procedures. The 5-μm histological sections were dyed using hematoxylin–eosine (HE) for histopathological examination. The anatomopathological diagnosis of the degree of spinal cord injury was established by microscopic appearance, and the definition of lesion types was based on the length of the spinal cord injury.

2.7. Statistical analysis

The sleep pattern variables for the 2–12-h light–dark periods—with periodic measurements level, normal distribution and similar variances were analyzed using the two-factor ANOVA test (GROUP factor: SHAM and SCI; DAY factor: baseline, D1, D2, D3... D7), with repeated measures for the day factor. The Tukey test was applied as necessary. The comparison between the SHAM and SCI groups as to the number of animals presenting movement of the limbs was made through the exact Fisher test each day. All analyses used a 0.05 significance level and results were presented as means ± standard deviation.

3. Results

3.1. Histopathological study

Histopathological examination revealed morphologic alterations in all spinal cord submitted to surgical procedure. Four different sets of length of lesion were seen in the spinal cord, as follows:

- Group 0: no histopathological alterations (SHAM group);
- Group I: lesion predominantly affecting central part of dorsal column (2 spinal cord);
- Group II: lesion affecting predominantly the entire dorsal column (3 spinal cord);
- Group III: lesion affecting predominantly hemimedula, including the whole dorsal column (3 spinal cord);
- Group IV: lesion affecting almost all the spinal cord, including the whole dorsal column (3 spinal cord) (Fig. 1).

3.2. Limb movements

Ten of the 11 rats in the SCI group presented limb movement during Non-REM sleep. The numbers of animals presenting limb movements were statistically significant on days 5, 6 and 7 (p < 0.05—Fisher) when compared to baseline recording and the SHAM group. The animals in the SHAM group did not present limb movement during the 8-day recording period (Fig. 2).

The average duration of limb movements during sleep was 3–9 s. Some rats presented isolated movements, while others presented series of movements during some sleep periods. The number of series of movements was 5 per sleep episode, and they were not necessarily followed by an arousal. It must be emphasized that the rats presented a polyphasic sleep pattern and this made it hard to estimate values for a paw-movements per hour index.

The spinal cord injury rats showed the same pattern in the four different types of injury groups for EMG records; sleep pattern and paw movements when the injury was sufficient to cause alterations in sleep patterns.

3.3. Sleep parameters

Sleep Efficiency levels observed showed a decrease of Total Time of Sleep on the first day after spinal cord injury in the light period recording for the SCI group compared with baseline recording or when compared with the SHAM group (p < 0.05—Tukey). The dark period recording showed higher sleep efficiency after spinal cord injury in the SCI group on days 3 and 5 when compared with baseline recording and on days 3 and 4 when compared with the SHAM group (p < 0.05—Tukey) (Fig. 3).

There was an increase in Total Wake Time (TWT%) in the light period recording on the first day after spinal cord injury of the SCI group compared with the baseline recording and compared with the SHAM group (p < 0.05—Tukey). The dark period recording showed a decrease in TWT% for the SCI group on days 3 and 5 when compared with the baseline recording and a decrease
Fig. 1. Outline of the four groups with different lengths of histopathological lesion of spinal cord (hematoxylin–eosine (HE) for histopathological examination). (A) Spinal cord without histopathological alterations (SHAM group). (B) Spinal cord with lesion affecting part of dorsal column. (C) Spinal cord with lesion affecting entire dorsal column. (D) Spinal cord with lesion affecting hemimedulla and including dorsal column. (E) Lesion affecting almost all the spinal cord, including the whole dorsal column.
on days 3, 4, 5 and 6 when compared with the SHAM group ($p < 0.05$—Tukey).

Non-REM sleep showed a reduction in the light period on the first day after spinal cord injury for the SCI group when compared with baseline recording ($p < 0.05$—Tukey). During the dark period recording, the SCI group showed an increase in Non-REM% on day 5 when compared with baseline recording and on days 3, 4, 5 and 6 when compared with the SHAM group ($p < 0.05$—Tukey).

PS was reduced on the first day after spinal cord injury in the light period from the SCI group when compared with baseline recording and when compared with the SHAM group ($p < 0.05$—Tukey). The dark-period recording did not show statistically significant difference (Fig. 4).

The light and dark period recording variables for number of arousals (NA) and latency to sleep (LAT) did not present statistically significant difference.

4. Discussion

Our results showed that after sectioning the spinal cord, the rats began to present spontaneous limb movements similar to those observed in PLM patients during sleep. The T9-level spinal cord injury led to limb movements in 90% of the rats studied. These movements were detected during the light period (7:00h–19:00h), when rats showed greater sleep efficiency. The control group rats were not submitted to spinal cord injury and did not show spontaneous movements during the 8-day recording period.

After the first day of spinal cord injury, there was a reduction in sleep efficiency during the light period recording (12-h light) for SCI group rats compared with baseline recording and with the SHAM group. Sleep efficiency during the dark period (12-h dark) increased after the spinal cord injury. On analyzing the light and dark periods together over a 24 h period, it was observed that sleep efficiency in the SCI animals was higher than in the SHAM group and returned to near baseline values at the end of the 7-day period.

Alterations in motor activity and behavioral patterns in SCI rats were studied by Mills et al. [12], who analyzed exploratory behavior and chronic central pain. Their results showed that these behaviors could be used to measure and evaluate chronic central pain in SCI rats.

The SCI group rats presented altered sleep efficiency, which was greater in dark-period sleep than the SHAM group's. This maybe due to the fact that these animals showed less motor activity after spinal cord injury and there was a period of adaptation to the new behavior pattern.

In an earlier attempt to develop an RLS model in the rat, two studies showed paw movements in rats, with sub cortical injuries in the A11 dopaminergic nucleus leading to higher levels of locomotion. The results suggested that the motor restlessness observed in the rats might be similar to the characteristics described by the Restless Legs Syndrome [13].

In another study, Baier et al. [2] evaluated the effect of age on the PLM phenomenon. Spontaneous occurrence of PLM in animals, young people and the elderly has been studied, as has the effect of the antidopaminergic substance haloperidol on this kind of movement in older animals. The older animal group showed an exacerbated level of spontaneous limb movements during sleep in relation to the young group. No difference in number of movements during sleep was detected in older animals after administration of haloperidol. A possible explanation of this contradictory finding...
could be the large night-to-night variability of PLM occurrence rather than a pharmacological effect.

It must be emphasized that the limb movement pattern detected in our animal model shows several similarities with the parameters described in PLM [1]. The altered EMG lines in our study were simultaneously checked on video and this enabled us to detect movements more precisely. These limb movements followed the same pattern. In other words, all rats presented the same type of movement (flexing following by extension), whether or not followed by an arousal.

Our histopathological study divided the rats into four different spinal cord injury groups, obtained using the same procedures, depending on the length of spinal cord affected in GI, GII, GIII and GIV. There was lesion of the posterior spine and gracilis and cuneate fascicles in all these groups.

In the spinal cord the ascending spinal column paths are important for fine tactile discrimination and proprioception, and establish feedback between muscles and joints, which is essential for control of motor functions and skills. About 25% of the spinal axions are propriospinal, i.e. are fibers that originate and terminate in the spinal cord [18].

We do not know which physiopathogenic mechanisms or structural alterations generate spontaneous movements during sleep, such as PLM or myoclonias.

The literature points to the absence of cortical potentials preceding PLM, thus excluding direct involvement of the cerebral cortex in its origin [11,19].

Findings reporting some abnormal medulla conditions have been described in previous articles [4,6,9,20]. On this basis, may we state that the fact of there being some morphofunctional alteration in the medulla is enough to trigger an abnormal pattern of movement during sleep? Yokota et al. [20] showed that myelopathy, cervical spondylosis and spinal vascular accident patients presented PLM during sleep. Work by De Mello et al. [4,6] has reported the incidence of PLM in individuals with full sectioning of the spinal cord detected by neuro-imaging. Provini et al. [14] suggested that activation (or disinhibition) of enervated skeletal muscles at medullar levels L4–S1 and to a lesser extent at C6–C7, is involved in generating PLM.

This study has shown that lesion of the gracilis and cuneate fascicles were sufficient to generate this pattern of
movement during sleep, regardless of the length or degree of histopathological lesion. The movements persisted even when lesion of the ventral horn was detected, with impairment of the motor neurons—together with the lesion of the dorsal column. However, motor lesion did not interfere with or modify the pattern of movement. Extent of lesion was not a crucial factor determining whether limb movements were presented during sleep. Our results point to possible involvement of the gracilis and cuneate fascicles in generating spontaneous movements during sleep.

Involvement of the corticospinal pathway cannot be ruled out. Severance or blockage of the latter by the lesion could be generating these movements, since this descending motor path has a dense nerve termination in laminae 3–7 of the spinal horn and low-density terminals in the ventral horn. The motor neurons—together with the lesion of the ventral horn has a dense nerve termination in laminae 3–7 of the spinal horn and low-density terminals in the ventral horn [18].

Another hypothesis for the involvement of the dorsal column in movements during sleep is the topographical location of the dopaminergic neurons in the dorsal column of the spinal cord. Therefore, lesion of these neurons may be prompting the alterations observed in the rats. The dopaminergic system cells are located periventricularly in the dorsal hypothalamus and caudal thalamus, and their nerve endings have their synapses in the dorsal horn at all levels of the spinal cord and around the preganglionic sympathetic neurons in the thoracolumbar spinal cord. The data now available favor the participation of the spinal dopaminergic system in pain modulation and autonomic and motor responses. Dysfunction of spinal dopaminergic neurons may be involved in the pathophysiology of certain conditions, such as Parkinson’s disease [10].

Accordingly, our data emphasize the importance of the spinal cord in causing PLM, regardless of cortical involvement. Therefore, spinal cord injury may be causing propriospinal stimuli capable of altering the pattern of functioning of the spinal cord motor cells.

We believe this animal model has the potential to contribute to an enhanced understanding of the origin of PLM.

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