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Cırcadıan changes ın cortıcal excıtabılıty ın restless legs syndrome

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ABSTRACT

Various investigations have revealed a widespread and somewhat controversial pattern of cerebral, cerebellar and brainstem involvement in the pathophysiology of restless legs syndrome (RLS). However, several studies which investigated functional or structural aspects indicated cortical involvement in RLS. In this study, we aimed to analyze circadian changes of cortical excitability in idiopathic RLS patients by means of transcranial magnetic stimulation (TMS). Eleven idiopathic RLS patients and eight healthy age and sex matched subjects were investigated using single-pulse TMS and motor nerve conduction studies during early afternoon when there were no symptoms and late at night (22:00–23:00) when the symptoms reappeared. Central motor conduction time, latencies and amplitudes of scalp and cervical motor evoked potentials, resting and active motor thresholds, and cortical silent period were measured. Measured parameters were similar between RLS patients and healthy subjects during the daytime. At night, cortical silent periods tended to shorten, and motor thresholds tended to decrease in the RLS group, whereas in controls they tended to increase.

At night, active motor-threshold measurements were significantly lower in the RLS group ($28.5 \pm 6.2\%$ vs $40.4 \pm 8.4\%$, $p = 0.006$). Therefore, we propose that in patients with RLS, conduction along the motor corticospinal axons is normal, with the possible loss of subcortical inhibition at nighttime.

1. Introduction

Restless legs Syndrome (RLS) consists of sensory and/or motor symptoms that are relieved by movement and are more prominent in the late afternoon or in the evening. Although there are some diagnostic methods based on detection of involuntary movements during rest, an exact diagnosis depends on clinical criteria [1]. One clinical criterion is a circadian change in symptoms. Various studies have revealed a widespread and somewhat controversial pattern of cerebral, cerebellar and brainstem involvement in the pathophysiology of RLS [3].

However, several studies investigating functional or structural aspects have indicated cortical involvement in RLS. [2]. Recently, using event-related beta and mu (de)synchronization, Tyvaert and colleagues [3] detected a modification in rhythm reactivity in RLS patients during the evening compared with morning tests or with healthy controls and concluded that this finding was consistent with the involvement of the sensorimotor cortex in primary RLS.

Transcranial magnetic stimulation (TMS) is a non-invasive method that can be used for the evaluation of cortical excitability [4]. Typical TMS parameters for studying cortical excitability are the cortical active motor threshold (aMT) and resting motor threshold (rMT) and the cortical silent period (CSP). Previously, CSP was found to be decreased in naive RLS patients [5] and it was shown to be reversed after cabergoline treatment [6] although Kütükçü and colleagues [7] failed to show any change in the corticomotoneural excitability of RLS patients.

This difference in findings may originate from the fact that those studies did not consider circadian effects on symptoms. Therefore, we sought to analyze circadian changes in cortical excitability in idiopathic RLS patients by means of TMS.

2. Patients and methods

Patients with RLS symptoms admitted to The Istanbul University Cerrahpasa School of Medicine, Neurology Outpatient Clinic from January 2008 to July 2008 comprised the study population. All patients were assessed by the same sleep specialist (D.K.) and RLS was diagnosed according to International RLS Study Group criteria.

2.1. Patients and controls

Eleven idiopathic RLS patients (54.5% male) with a mean age of 50.2 ± 13.3 years were included in the study. The range of disease duration was 1 to 20 years. Mean frequency of symptoms was 5.5 ± 2.3 days/week and 20% had upper extremity symptoms in addition to lower extremity involvement. Patients with previous treatment for RLS, history of any systemic, psychiatric, or neurological disease, drug/substance abuse that may interfere with electrophysiological

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Table 1
Clinical features of RLS and control groups.

	RLS group	Control group
Mean age (±SD, y)	50.2 ± 13.3	48.1 ± 16.9
Age range (y)	23–66	23–70
Sex (F/M)	5/6	4/4
Mean age at disease onset (±SD, y)	37.7 ± 11.7	–
Age range at disease onset (y)	20–54	–
Family history (%)	10	–
Upper extremity symptoms (%)	20	–
Frequency of symptoms (day/week)	5.5 ± 2.3	–

(RLS, restless legs syndrome; y, year; F, female; M, male).

investigations (depression, peripheral neuropathy or any systemic disease which may lead to peripheral neuropathy, use of antidepressant drugs) or any systemic or neurological disease in which electrophysiological investigations may have contraindications (i.e. cardiac pacemakers) were excluded. All patients were naive to dopaminergic and antidepressant drugs as well as to opioid agonists and all had ongoing rhythmicity. Results were compared to those of eight healthy age- and sex-matched subjects (50% male; mean age, 48.1 ± 16.9). Demographic and clinical features are shown in Table 1.

All patients gave informed consent. The study was approved by Ethical Committee of Istanbul University Cerrahpasa School of Medicine and was supported by the Istanbul University Research Project Unit.

2.2. Methods

2.2.1. Clinical assessment

All patients and healthy controls underwent detailed clinical interviews neurological examinations and their medical records were analyzed retrospectively for the presence of medical, neurological, or psychological diseases or any other accompanying sleep disorders including periodic limb movement disorder (PLMD), insomnia, obstructive sleep apnea syndrome (OSAS). All RLS patients and healthy volunteers with symptoms of sleep disorders underwent polysomnography. There was no accompanying PLMD and/or insomnia in either groups. Frequency and presence of OSAS was similar between the two groups. Routine biochemistry investigations including serum ferritin, vitamin B12, folate, urea and glucose levels were performed in the RLS group to exclude secondary factors.

2.2.2. Electrophysiological examinations

Single pulse-TMS and motor nerve conduction studies and F-wave investigations were performed under similar conditions in a quiet room using Neuropack Sigma MEB-9100 (Nihon Kohden, Tokyo, Japan).

Motor conduction studies of the ulnar nerve were performed first using the first dorsal interosseus (FDIO) muscle. Compound motor action potentials (CMAP) were recorded by Ag–AgCl surface electrodes using the standard belly-tendon montage. Supramaximal electrical stimulus was applied at the level of the wrist and the elbow. Amplitude sensitivity was 5 mV; analysis time was adjusted to 2 ms/div. The filter settings were 5 kHz high cut and 10 Hz low cut. Responses (20 F) to supramaximal stimulation were obtained using the same settings, except for amplitude sensitivity and analysis time, which were adjusted to 0.1–0.2 mV and 10 ms/div, respectively. Subjects were subsequently evaluated by TMS. Single pulses were generated by MAGSTIM 200^2 monophasic TMS equipment (Medelec, UK) and a standard 90 mm circular stimulation "coil" (3193–00). All tests were performed twice on each subject: during early afternoon, when there were no symptoms, and at night (22:00–23:00) when symptoms reappeared. Because stimuli presented during the initial investigation might have an impact on excitability, daytime and nighttime investigations were performed on different days, randomly. All recordings were performed while subjects were in a supine position. To normalize vigilance during TMS sessions, all subjects were asked by the investigator to remain alert, with open eyes, and to count the stimuli. The stimulus number was reported by the subjects and confirmed at the end of each session. The FDIO muscle was used as the index muscle for TMS investigations. Ag–AgCl surface electrodes were placed over the FDIO according to the standard belly-tendon montage and the stimulus was applied over the nuchal midline (cervical MEP) or over the vertex, specifically of the hand area of the contralateral side (scalp MEP) via circular coil at an intensity of 90%. Analysis time was adjusted to 10 ms/div and amplitude sensitivity was 200 mV. The filter settings were 3 kHz high cut and 20 Hz low cut. Stimuli were repeated eight times with an interstimulus interval minimum of 20 s and minimal onset latency (ms) and peak-to-peak amplitudes (mV) were measured.

Central motor conduction time (CMCT; ms) was measured by two different methods: 1. $(F+M-1)/2$ (Central motor conduction time-F, CMCT-F); or 2. minimum scalp MEP latency–minimum cervical MEP latency (Central motor conduction time-C, CMCT-C). In the first formula, F stands for the shortest F latency among the 20 different F latencies and M represents for the M latency. When neuron is stimulated antidromically, there is a delay time represented by 1 in the formula [8].

The intensity that generated a response amplitude of 100 mV for 50% of eight stimuli was accepted as rMT. aMT was estimated while a subject was performing a slight (approximately 10–25% of maximum) contraction. Initially, each subject performed a maximal contraction, and we calculated the envelope amplitude during maximal contraction. Meanwhile, the subject was given audiovisual feedback about the characteristics of maximal contraction. Afterward, the subject performed a series of contractions until the envelope amplitude reached 10–25% of that obtained during maximal contraction. This procedure was simplified using a dynamometer. To stabilize at the 10–25% contraction recurring controls were done by way of dynamometer. The intensity generating a response with amplitude of 50 mV in 50% of eight stimuli was accepted as aMT. Amplitude sensitivity during threshold investigations was 0.2 mV.

CSP was recorded for eight stimuli at by 140% of the intensity that generated aMT. Analysis time was 10 ms/div and amplitude sensitivity was 1 mV.

2.3. Statistical analysis

Latency, duration, and amplitude of responses were measured by the position of the cursor, whereas area was calculated automatically by the EMG instrument after marking the range of responses for each individual. Data were pooled to obtain mean values and standard deviations (SD). Distal motor latency (ms), compound muscle action potential amplitude (mV), area (μV ms) and motor nerve conduction velocity (m/sc) of the ulnar motor response as well as persistence (%), minimum latency (ms), mean amplitude (μV) of the F wave and F/M ratio were measured. For the TMS investigations, cervical and scalp MEP latency and amplitude, aMT, rMT, duration of CSP, CMCT-C, and CMCT-F were measured. To evaluate circadian variation, comparisons were made between daytime and nighttime values of each group. As the distributions of these data were non-homogeneous, nonparametric Wilcoxon tests were executed for these comparisons. Nighttime values and daytime values for the patient and control groups were compared using Mann–Whitney *U*-tests.

3. Results

3.1. Clinical assessment

No PLMD or insomnia was observed in either the RLS or control group. The frequency and presence of OSAS was similar between the groups.

Table 2
Comparison of scalp and cervical TMS parameters between RLS patients and healthy volunteers.

	DT RLS	DT control	p: Mann Whitney	NT RLS	NT Control	p: Mann Whitney	DT vs NT for RLS p: Wilcoxon	DT vs NT for control group p: Wilcoxon
Scalp MEP								
Latency (ms)	22.2 ± 3.4	21.2 ± 1.2	0.741	20.9 ± 2.6	20.7 ± 1.9	0.869	0.139	0.352
Amplitude (mV)	6.0 ± 4.1	5.1 ± 2.4	1.000	3.2 ± 2.2	4.6 ± 1.9	0.155	0.779	0.575
Cervical MEP								
Latency (ms)	13.9 ± 2.2	13.7 ± 1.1	0.803	14.2 ± 1.9	13.7 ± 1.4	0.457	0.109	0.778
Amplitude (mV)	5.6 ± 5.1	3,6 ± 3.3	0.564	3.7 ± 3.3	4.5 ± 3.1	0.477	0.790	0.575
aMT (%)	37.8 ± 14.7	36.0 ± 6.7	0.859	*28.5 ± 6.2*	*40.4 ± 8.4*	*0.006**	0.089	0.205
rMT (%)	59.4 ± 14.9	59.1 ± 12.7	0.752	53.7 ± 18.9	60.4 ± 9.4	0.316	0.398	0.624
CSP duration (ms)	47.2 ± 30.8	44.6 ± 35.1	0.934	45.5 ± 30.1	56.2 ± 49.1	0.509	0.328	0.484
CMCT-C (ms)	8.2 ± 3.2	7.5 ± 0.8	0.836	6.7 ± 1.4	7.1 ± 1.5	0.159	0.142	0.400
CMCT-F (ms)	7.4 ± 3.1	7.3 ± 2.9	0.741	6.2 ± 1.6	5.6 ± 2.0	0.247	0.197	0.161

(All parameters are presented as mean ± SD.).
(TMS, transcranial magnetic stimulation; RLS, restless legs syndrome; DT, daytime; NT, nighttime; MEP, motor evoked threshold; aMT, active motor threshold, rMT, resting motor threshold; CSP, cortical silent period; CMCT-C, Central motor conduction time-C; Central motor conduction time-F, CMCT-F); *p<0.05.

3.2. Electrophysiological investigations

Ulnar nerve motor responses in all patients and controls showed normal latencies, conduction velocities and amplitudes in both daytime and nighttime investigations. Normal F responses were also recorded in all participants. There were no significant differences in latency, amplitude and CV of motor responses or in minimum latencies of F waves, between patient and control groups in both daytime and nighttime measurements. F wave persistence and amplitude were also similar between groups.

In both daytime and nighttime investigations, cervical and scalp MEP latencies and amplitudes, rMT, duration of CSP, CMCT-S, and CMCT-F were similar between RLS and control groups (Table 2). Comparison of day- and nighttime investigations in each group failed to demonstrate any differences., only nighttime aMT was lower in RLS group compared with controls (28.5 ± 6.2 vs 40.4 ± 8.4, p = 0.006). CSP had a tendency to decrease in both groups and there were no significant differences between groups.

4. Discussion

In this study we have demonstrated that at night rMT, aMT and duration of CSP tended to decrease in RLS patients whereas healthy volunteers showed a trend to increase and circadian decrease of aMT reached statistical significance. Previously, shortening of CSP, decreased aMT or rMT, decreased intracortical inhibition (ICI), and increased intracortical facilitation (ICF) have all been reported in RLS. To our knowledge, this is the first study aiming primarily to investigate circadian changes in single-pulse TMS parameters in RLS.

MEP amplitude demonstrates synchronization and the number of alpha-motoneurons that finally discharge in response to cortical shock and CMCT represents integrity of motor corticospinal axons. As both of these measures were similar and were within the normal range in both the RLS and control groups, spinal integrity and excitability appear to be normal in RLS patients. Motor threshold is expressed as the minimum stimulus intensity capable of evoking MEPs in the target muscle at rest (rMT) or during voluntary preactivation (aMT). During rest, recordings started with stimuli that generated maximum MEP amplitude, and stimulus intensity was then decreased by 2%. Although the threshold is affected by many stimulus characteristics it may be used as an index of cortico-motoneuron system excitability [9]. Pharmacological studies suggest that it reflects, at least in part, the membrane permeability ion channels of the cortical motor neurons [10]. Relaxed thresholds largely reflect the excitability of presynaptic cortical axons [9]. The physiological meaning of aMT is even more complex, because during voluntary contraction, excitability of the entire cortico-motoneuron connection is enhanced and postsynaptic phenomena are likely to play a more important role [10]. Our only significant finding was decreased aMT in RLS during the night with a trend toward the circadian decreases of MT and CSP. We may speculate that this finding may reflect subcortical interactions and excitability rather than cortical excitability. Other studies reporting shortened ICI and increased ICF also indicated subcortical–supraspinal disinhibition [11,12]. However, we should keep in mind since we could not conduct multivariate analysis due to low number of patients.

The CSP represents an electromyographic silence of variable length that follows the MEP when the target muscle is preactivated [9]. Many segmental and suprasegmental inhibitory effects may contribute to this phenomenon. Segmental phenomena would play a role in the early portion of CSP, whereas the later portions are more likely to be supraspinal [13], depending on a lack of excitatory drive onto the spinal alpha-motoneurons. The cortical silent period has been used as an index of cortico-motoneuron excitability. Previously, CSP was reported to be shortened in RLS. This was attributed to disturbance of the subcortical dopaminergic system and proposed to reflect disinhibition of inhibitory pathways [5]. Although subsequent reports commented that in this study, patients were using dopaminergic agents shortly before performing tests which may result in a rebound increase at the time of testing [12], shortened CSP seemed to be a persistent finding and it was reversed after dopaminergic treatment [6]. In a study, as a secondary analysis, it was observed that in particular patients CSP demonstrated circadian changes, although the group data did not confirm [14].

Although, we found that CSP and rMT of RLS patients tended to shorten at night, in contrast to observations in healthy volunteers, we did not observe significant circadian change. aMT was significantly lower at nighttime in RLS group, however, we could not conduct multivariate analysis because of the small group size. Moreover, we also failed to find significant differences between RLS patients and healthy controls in contrast to previous studies which may result from limitations of our study. Although all of our patients were drug-naïve, their clinical features were heterogeneous, some had longer durations of disease up to 20 years and some had daytime symptoms. Other limitations were the small number of patients and the absence of paired-pulse TMS parameters. Frequent presence of systemic, psychiatric or neurological disease and frequent use of antidepressant drugs decreased the eligibility and subsequently number of RLS patients included in this study. However, this criterion allowed for the analysis of excitability distinctly in RLS. Investigations involving upper extremity muscles may appear to be another limitation of our study. However, CSP was previously shown to be shorten when recording over AT or APB muscles [7] and technical difficulties regarding MEP recordings over lower extremity muscles support the accuracy of our method.

RLS is a movement disorder in which cortical, subcortical, brainstem or spinal generators have been suggested to be involved. We propose

that in patients with RLS conduction along motor corticospinal axons is normal with possible loss of subcortical inhibition at night.

Conflict of Interest

None declared.

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