Circadian Change in Blink Reflex Recovery in Restless Legs Syndrome

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ABSTRACT

Introduction: Restless legs syndrome (RLS) is associated with dysfunction of the dopaminergic systems in the pathways that specifically link the sensory input and motor output. Keeping in mind that clinical symptomatology in RLS and cerebrospinal fluid dopamine concentrations in healthy individuals show changes throughout the day, we hypothesized that excitability of the related pathways increases during the night in RLS, and in the present study, we aimed to analyze our hypothesis by the assessment of blink reflex (BR) recovery.

Methods: Eleven patients with primary RLS and eight age- and gender-matched healthy subjects were included in the study. All participants underwent detailed interviews and neurological examinations. BR responses were recorded after single and paired supraorbital stimulation during the early afternoon and late at night. For double stimulation, interstimulus intervals (ISI) of 100, 300, and 500 ms were used. Daytime and nighttime investigations were separately compared between the patient and control groups (between-group analyses).

In-group analyses were conducted between daytime and nighttime investigations of the patient and control groups.

Results: BR responses to single stimuli were normal in all participants at all sessions. R2 recovery was the highest in the patient group during nighttime investigations. In-group analysis showed a reduction of R2 recovery during the night in healthy subjects. R2 recoveries at ISIs of 300 and 500 ms at nighttime were higher in RLS patients but did not reach statistical significance.

Conclusion: The BR circuit is less excitable during the night in healthy individuals, whereas the reduction of excitability is lost in RLS. Despite the limited number of included subjects, we suggest that the normal circadian modulation of the BR circuit is lost in RLS.

Keywords: Blink reflex, blink reflex recovery, brainstem excitability, circadian rhythm, restless legs syndrome

INTRODUCTION

Restless legs syndrome (RLS) is characterized by an irresistible urge to move the legs, usually accompanied by an abnormal sensation. Symptoms are generally prominent during the early nighttime period (1). This characteristic feature persists even after the appearance of daytime symptoms with progression of the disease.

Pathways that specifically link sensory input and motor output are hypothesized to underlie the pathophysiology of RLS (2,3). Several studies have supported this idea: deficiency in dopaminergic systems of the putamen and substantia nigra in a postmortem study (4), reversibility of clinical and electrophysiological findings after dopaminergic treatments (5), and functional imaging studies (6) suggest the involvement of the dopaminergic pathways at the level of the brainstem and basal ganglia.

Recovery of the blink reflex (BR) after double stimulation reflects the excitability of BR, which is shown to be under dopaminergic control and enhanced in disease states such as Parkinson's disease (PD) (7,8,9,10,11) or periodic limb movement disorders (PLMD) (12). Previously, only few studies investigated BR in RLS and found no change (13); however, there are no studies regarding the circadian change in BR excitability.

Given the information that the clinical symptomatology in RLS changes throughout the day and cerebrospinal fluid dopamine concentrations show circadian changes in humans (14), we hypothesized that the functional activity in the dopamine system decreases during the night in RLS and aimed to analyze our hypothesis by the assessment of BR recovery.

METHODS

Participants: All our drug-naive RLS patients who were diagnosed according to the International RLS Study Group criteria (1,15) by a senior neurologist experienced in sleep disorders between February 2008 and August 2008 underwent detailed interviews, neurological examinations, routine biochemical investigations, including serum ferritin, vitamin B12, folic acid, urea, and glucose, and polysomnography when their history indicated symptoms of another sleep disorder (PLMD, insomnia, or obstructive sleep apnea syndrome [OSAS]).

Patients with secondary RLS, previous treatment for RLS, history of any systemic, psychiatric, or neurological diseases, or drug/substance abuse that could interfere with the electrophysiological investigations, or a history of any systemic or neurological diseases in which electrophysiological investigations may be contraindicated (i.e., cardiac pacemakers), or insomnia related to causes other than RLS and PLMD were excluded.

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Eleven patients with primary RLS (54.5% male; mean age, 50.2±13.3 years) fulfilled the criteria. For comparisons, eight age- and gender-matched healthy subjects (50% male; mean age, 48.1±16.9 years) were included.

All the participants gave informed consent. This study was approved by the Institutional Review Board and was supported by the Istanbul University Research Project Unit.

Electrophysiological examinations: All the investigations were performed before the start of dopaminergic medications under similar conditions in a quiet room while the patients were in a supine position using the Neuropack Sigma MEB–9100 instrument (Nihon Kohden, Tokyo, Japan) during the early afternoon (13:00–13:30) when there were no symptoms, and late during the night (22:00–23:00) when symptoms reappeared. Daytime and nighttime investigations were performed randomly on different days. Briefly, the first patient who initially underwent daytime investigations underwent nighttime investigations in the next week and vice versa for the second patient.

Blink reflex responses were recorded over the orbicularis oculi (O.oc) muscles using Ag-AgCl surface electrodes following cutaneous bipolar electrical stimulation of the supraorbital nerve. An active recording electrode was placed over the middle part of the inferior O.oc, while a reference electrode was located 2 cm lateral to the rima oculi. The ground electrode was placed on the forehead. A single electrical stimulus with a duration of 0.2 ms and intensity of 2.5–3 times the R2 threshold (8–14 mA) was applied percutaneously to the supraorbital nerve at its exit from the supraorbital foramen. The stimulus was given randomly as five consecutive bursts with a minimum interval of 20 seconds to prevent habituation. The filter settings were 3 kHz high-cut and 20 Hz low-cut. Analysis time was adjusted as 10 ms/div, and the amplitude sensitivity was 200 μV. Reflex responses were accepted when there was an evident response starting with a sharp negative deflection. Onset latency (ms), amplitude (μV), and the area (ms μV) of the R1, R2, and R2C responses following each of the five stimuli were measured, and the mean values were calculated.

Latency, duration, and amplitude of the responses were measured using cursors, whereas the area was calculated by an electromyography instrument automatically after marking the response breadth. Data were pooled to obtain the mean values and standard deviations. To obtain the BR recovery, electrodes were placed in a manner similar to that described in the BR methodology. Constant current paired stimuli with the same stimulus characteristics as the single stimulus were delivered at interstimulus intervals (ISI) of 100, 300, and 500 ms. We obtained five consecutive responses, and all the setting parameters were the same, except for the analysis time, which was adjusted to 50 ms/div during stimulation with ISIs of 100 and 300 ms and to 100 ms/div during stimulation with an ISI of 500 ms. We measured the areas (ms μV) of the R2 responses following the first stimulus (conditioning) and second stimulus (test). We calculated the percentage of excitability recovery using the following formula:

100 × (the area of the R2 response to the test stimulus/the area of the R2 response to the conditioning stimulus).

Statistical analysis
Mean values of the latency, amplitude, and area of BR responses to a single stimulus and the mean percentage recovery of the response to the test stimulus with paired stimulation at ISIs of 100, 300, and 500 ms were compared. First, comparisons were made between daytime and nighttime values within each group (in-group comparisons). As the distribution of these groups was heterogeneous, the nonparametric Wilcoxon test was used for this comparison. Second, nighttime and daytime values of the patient and control groups were compared using the Mann–Whitney *U*-test (between-group comparisons). Because there was no difference between the right and left sides, only right side data were shown and used for further analyses.

Response recovery was assessed by the Friedman test. A post-hoc Dunn's test was used to identify significant differences. An R2 recovery index was

calculated as the mean of the R2 values at ISIs of 100, 300, and 500 ms and an index of each group is presented in a boxplot graph.

Patients were grouped according to disease duration (<5 years and ≥5 years), presence of upper extremity symptoms, presence of daytime symptoms), and R2 recoveries were compared between groups using the Mann–Whitney U test. A p value <0.05 was considered to be statistically significant.

RESULTS

Clinical findings: Eleven patients were included in the study (mean age: 50.2±12.6, M/F: 5/6). The range of disease duration was 1–20 years. The mean frequency of symptoms was 5.5±2.3 days/week. All the patients had lower extremity symptoms, and 20% had upper extremity involvement. Six patients had daytime symptoms. Only one had family history. The frequency and presence of OSAS were similar between the two groups.

Electrophysiological findings: R1 and R2 responses to single supraorbital nerve electrical stimuli were normal and similar in all healthy subjects and in patients in both the in-group and between-group comparisons.

Between-group comparisons showed that R2 recoveries at ISIs of 100, 300, and 500 ms during the daytime investigations were similar between the patients and healthy subjects. R2 recoveries at all the ISIs during the nighttime investigations were higher in the patient group compared to in the healthy subjects (Figure 1); however, the differences were not statistically significant.

In-group analysis showed a reduction of R2 recovery during the night in healthy subjects, whereas R2 recovery increased in RLS patients. The upper limit of the R2 recovery index was also the highest in the patient group during the daytime, and comparisons of the daytime to nighttime R2 recovery index showed that it tended to increase in the RLS group despite the reduction in healthy subjects (Figure 2).

There were no significant relations between the R2 recovery and clinical findings like age, disease duration, family history, and presence of upper extremity symptoms. However, R2 recovery changed according to the presence of daytime symptoms. Patients with daytime symptoms had lower nighttime R2 recoveries compared to daytime R2 recoveries, whereas nighttime R2 recovery was higher in patients without daytime symptoms (Figure 3).

DISCUSSION
R1 and R2 responses to single supraorbital nerve electrical stimuli were normal and similar in all healthy subjects and in patients in both in-group and between-group comparisons. BR is an established electrophysiological investigation examining the functions of the brainstem in routine clinical practice. After supraorbital trigeminal electrical stimulation, two responses, the R1 and R2 components, were obtained. The afferent and efferent pathways are the ophthalmic branch of the trigeminal nerve and the facial nerve, respectively (16). The central generator of the R1 is located in the pons, whereas the R2 pathway involves numerous synapses bilaterally in the brainstem reticular formation from the pons to the medulla. Normal responses reflect integrity of at least the BR pathway in both nighttime and daytime investigations.

The pathway of the R2 component has multiple synapses and interneurons. Consequently, it is more prone to modulatory influences of suprasegmental structures, like specifically the superior colliculus, raphe nucleus, and basal ganglia (7,8). R2 recovery is the main electrophysiological method showing excitability of the interneurons and facial motor neurons. In our group, between-group comparisons showed that R2 recoveries at ISIs of 100, 300, and 500 ms during the daytime investigations were similar between the patients and healthy subjects. In contrast, the in-group analysis showed a reduction of R2 recovery during the night in healthy subjects, whereas it increased in RLS patients, suggesting an in-

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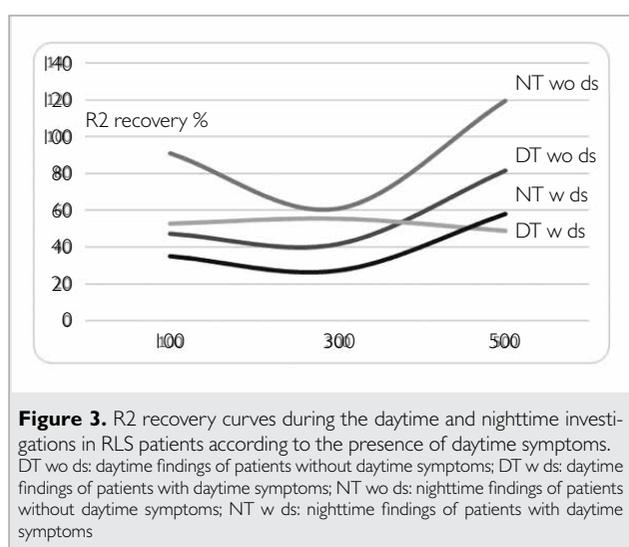
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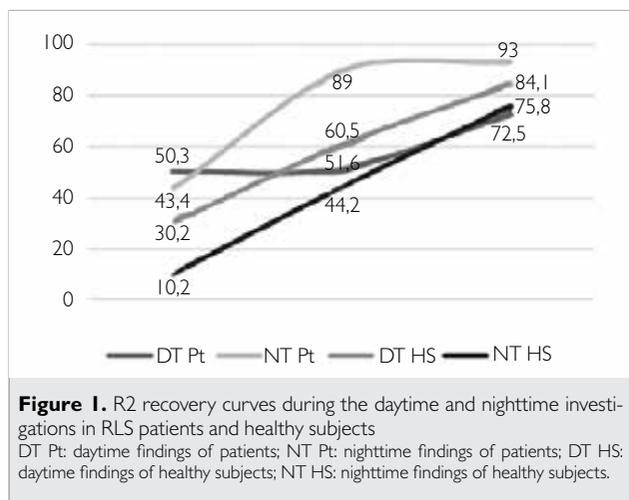
Figure 1. R2 recovery curves during the daytime and nighttime investigations in RLS patients and healthy subjects
DT Pt: daytime findings of patients; NT Pt: nighttime findings of patients; DT HS: daytime findings of healthy subjects; NT HS: nighttime findings of healthy subjects.
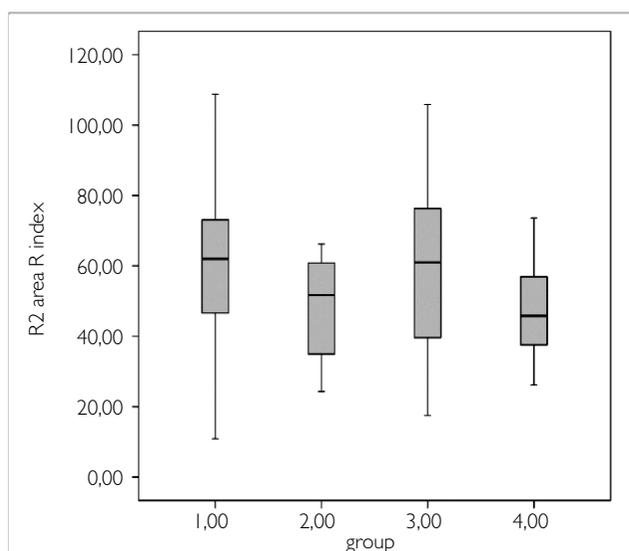
Figure 2. Boxplot graphs showing R2 recovery indices during the daytime and nighttime investigations in RLS patients and healthy subjects (y axis represents the R2 recovery index, group1: RLS patients, daytime investigations; group 2: healthy subjects, daytime investigations; group 3: RLS patients, nighttime investigations; group 4: healthy subjects, nighttime investigations)

Figure 3. R2 recovery curves during the daytime and nighttime investigations in RLS patients according to the presence of daytime symptoms.
DT wo ds: daytime findings of patients without daytime symptoms; DT w ds: daytime findings of patients with daytime symptoms; NT wo ds: nighttime findings of patients without daytime symptoms; NT w ds: nighttime findings of patients with daytime symptoms

creased reduction of excitability during the night in healthy subjects and a preserved high excitability during the night in RLS patients.

A reduction of dopamine in substantia nigra removes the inhibitory effect of the raphe magnus on BR, eventually leading to enhanced R2 excitability (7,8). Enhanced BR recovery has been reported in patients with basal ganglia dysfunction, like PD, or other types of Parkinsonism syndromes (9,10,11). It is also enhanced in PLMD, which has quite similar features to RLS and probably shares a similar pathophysiology with RLS. PLMD was reported to be related to impaired functions of dopaminergic projections on the brainstem reticular formation (12). Although the authors did not study the circadian changes, they showed an enhancement of BR excitability. Therefore, we also expected to see changes, even during daytime. In parallel to the widely-accepted hypothesis of RLS pathophysiology, changes in R2 excitability supports the function of dopamine in the development of RLS and the main neurotransmitter is supposed to be dopamine, which is supported by therapeutic options (17).

Dopamine has a circadian rhythm (14) and it is likely that the loss of circadian modulation of the dopamine circuitry underlies the RLS pathophysiology and appearance or worsening of symptoms during night. Functional neuroimaging studies, on the other hand, demonstrated conflicting results: lower, higher, or similar D2 receptor binding potentials (6,18,19). Among the various clinical differences between those studies resulting in the contradictory findings, one is probably ignorance of the circadian fluctuations.

As seen in the boxplot graphs, the values of both the R2 recovery index and the percentages of R2 recovery in the patient group changed in a broad range, which probably originates from the heterogeneous nature of our patient group. R2 recovery curves of patients with daytime symptoms were quite similar to those of the normal population. Although we strictly followed the clinical criteria and only included patients with preserved circadian rhythmicity, the presence of daytime symptoms led to heterogeneous findings. Therefore, we suggest that patients with daytime symptoms probably have different variations in the dopamine cycle.

Some limitations of our study should be mentioned. The patient group and healthy subjects included a limited number of individuals, which may have been responsible for the insignificant results despite the distinct differences on an individual basis. Therefore, our results should only be taken as a pilot study. The frequent presence of systemic, psychiatric, or neurological diseases and the frequent use of antidepressant drugs reduced the number of included patients with RLS. However, this criterion provided an analysis of distinct excitability in patients with RLS. Finally, BR excitability may be affected by several factors other than the diurnal rhythm, and the lack of supportive findings by dopamine transporter imaging may be another limitation.

In summary, our results suggest that a normal reduction of BR excitability during the night is lost in RLS. Consequently, considering the limited number of subjects, we suggest that the circadian change in modulation of BR excitability is lost in RLS and is related to circadian changes in the symptoms.

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