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# Neurophysiological and Morphometric Characteristics of Restless Legs Syndrome in Parkinson's Disease

**Olga A. Alenikova[1*], Nikita V. Alenikov[2] and Sergey A. Likhachev[1]**

*[1]Neurological Department, State Institution, Republican Scientific and Clinical Center of Neurology and Neurosurgery, Minsk, 220114, Belarus*
*[2]Health Care Agency "32nd City Clinical Health Centre", Minsk, Belarus*

**[*]Correspondence to:**
Dr. Olga A. Alenikova, PhD
Neurological Department, State Institution
Republican Scientific and Clinical Center of
Neurology and Neurosurgery
Minsk, 220114, Belarus
E-mail: alenikovaolga@gmail.com

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## Abstract

Restless Legs Syndrome (RLS) in patients with Parkinson's disease (PD) is seen as a non-motor symptom with no well-defined pathophysiology. RLS development involves dopaminergic diencephalon spinal tract dysfunction and supraspinal brain structural changes.

**Purpose:** The study was to investigate the neurophysiological disorders in PD patients with RLS using methods of somatosensory evoked potentials (SEP), blink reflex (BR) and sympathetic skin response (SSR). Estimation of daily parameters was done in relation with twenty-four hours Holter monitoring (HM) and brain structural alterations identified by MRI studies using voxel-based morphometry.

**Method:** 36 PD patients with RLS and 39 PD patients without RLS (RLS-) from 40-70 years age group and 30 healthy individuals with similar age group were examined twice a day at 8 am and 6 pm.

**Result:** In PD patients with RLS observed a significant increase in the amplitudes N20-P23, N13-P18, and N9-P8. Besides in the non-RLS group, the inter-peak interval (IPI) N9-N13, N11-N13 was shorter and the IPI N13-N20 was longer. PD patients with RLS have the predominance of sympathetic influence on the SSR. Thus a decrease in latency and an increase in the amplitude of second BR components are observed that indicate excessive polysynaptic reflex excitability at the brainstem level. HM detected the decrease in the circadian index that was a general trend of all the patients with PD, but a specific change in the time-domain parameters of heart rate variability also suggests autonomic dysfunction in the form of reduced sympathetic activity in PD patients with RLS. Disturbance of somatosensory processes and autonomic regulation have daily variability, which reflects the circadian pattern of RLS in PD. Structural changes in certain brain structures probably associate with functional neurophysiological abnormalities identified by mean voxel-based analysis of MTR images can be considered as predictors of RLS in PD patients.

## Keywords

Parkinson's disease, Restless legs syndrome, Somatosensory evoked potentials, Blink reflex, Sympathetic skin response, MRI voxel-based morphometry

## Introduction

Restless legs syndrome (RLS) is a chronic sensory-motor disorder characterized by sensory discomfort in legs, appearing or worsening during rest in the evening or night time, evoking the urge to move. This syndrome can be either

primary disorder, likely caused by a genetic predisposition, or secondary for other medical conditions. It can occur with such diseases as neuropathy, endocrine pathology, iron deficiency anemia, Parkinson's disease (PD), etc. [1, 2].

Several studies have reported that RLS is prevalent frequently in PD patients than in the general population [3]. However, RLS data is usual in PD patients which vary from 0 to 50%, depending on the study [4]. Moreover, many disputes about an etiological link between RLS and PD, and whether they share a common pathophysiology. Pathogenic mechanisms underlying the RLS are common with PD and closely related to dopaminergic insufficiency and iron metabolism disturbances in the structure of the extrapyramidal system [5-8]. Diencephalon spinal dopaminergic tract originating from the A11-A14 nuclei has received much attention as the potential anatomical site of dopaminergic dysfunction in RLS. The source of this tract is a group of dopaminergic neurons in the caudal hypothalamus and periaqueductal gray of the midbrain. Descending pathways target preganglionic sympathetic neurons in the intermediolateral nucleus (IML), dorsal horn regions responsible for afferent nerve processing, interneurons, and somatic motor neurons of the spinal cord in the RLS mechanisms [9-12]. Dopamine acted as an excitatory and inhibitory neurotransmitter in the spinal cord to regulate sensory, motor as autonomic functions [13]. Besides, other non-dopaminergic systems may also be involved in the pathogenesis of RLS. Locus Coeruleus (LC) contributes to sensory and motor functions control autonomic function through the projections to sympathetic and parasympathetic preganglionic neurons of IML and autonomic nuclei of the brainstem, the amygdala [14-17]. The periaqueductal gray (PAG) is also an important structure involved in the pathogenesis of the RLS. PAG is a poorly differentiated midbrain structure known to be involved in several key homeostatic neurobiological functions such as pain modulation, motor, cardiovascular, and other autonomic control [16, 17].

Disturbances of central somatosensory processes in RLS does not cause doubts. But it can be assumed that structural changes in the brain structures also involved in the pathogenesis of this disorder. In recent years, several imaging studies have focused on the evaluation of the central nervous structures in RLS patients. Although a series of magnetic resonance imaging (MRI) studies using various neuroimaging techniques have reported structural brain abnormalities in RLS, some questions remain unsolved. For example, Voxel-Based morphometry offers the opportunity to investigate subtle changes in gray and white matter volume that allows identifying morphological correlates of the CNS pathophysiological disorders *in vivo*, but the findings may differ between studies. Moreover, the complication may be the detection of specific brain changes characteristic of the RLS in PD patients who have widespread cortical and subcortical atrophy associated with Parkinsonism. Also, a certain complexity is the study of tissue changes in such brain structure as PAG due to their neuroimaging features [17, 18].

Another important aspect of the study of RLS are disorders in the systems that perform the circadian regulation of human biological functions. Clinical fluctuations of PD symptoms include diurnal changes in the level of motor activity [19], autonomic functions [20, 21], sleep-wakefulness cycle [22-24], visual functions [25] and the dopaminergic therapy efficiency [19]. Both sensory and motor symptoms also show a significant circadian rhythm in RLS, a peak displayed at a similar time in the evening or night. The key factors of circadian dysfunction can be retinal dopaminergic system involvement and melanopsin-expressing retinal ganglion cells (mRGC) degeneration which leads to «melatonin-dopamine» imbalance and distortion of information transmitted through the retinohypothalamic tract [26]. In general, rhythmic changes in dopaminergic and melatoninergic neurotransmission of different parts of the brain described in PD, but the reciprocal relationship between two neurotransmitter systems is one of the most significant in the retina. Changes in the functions of the mRGC involved in the non-visual function implication and contribute to the development of RLS in PD patients. This assumption confirmed by previous studies proving the evidence that the caudal PAG receives direct retinal innervations [27, 28]. Recently, a work carried out in genetically modified mice shows that sparse axonal terminations of mRGC are present in the rostral PAG [29]. This result suggests that light information transmitted by those PAG-projecting RGC may directly influence the neurons' activity in PAG and consequently affect the sensory, motor, and autonomic control.

Based on the above disorders of the sensory, motor, and autonomic systems at different levels in PD patients with RLS lead to changes in the functional state of the subcortical-brainstem-spinal systems and reflecting disturbances in spinal and brainstem reflexes. Since circadian disorders are based on RLS, it is not only the disturbance of reflexes but also the daily fluctuations of their parameters, which is objectified by various neurophysiological methods. For a better understanding of the nature of RLS, it is important to identify morphological correlates of the neurophysiological changes detected in PD patients with this syndrome.

So the purpose of our study was to investigate the neurophysiological disorders in PD patients with RLS using methods of somatosensory evoked potentials (SEP), blink reflex (BR) and sympathetic skin response (SSR) with estimation daily variability their parameters in relation to the results of the twenty-four hours Holter monitoring (HM) and brain structural alterations identified by MRI studies with using voxel-based morphometry.

## Methods

### Somatosensory evoked potentials

To assess the functional status of the somatosensory system in PD patients with RLS we used short-latency somatosensory evoked potentials (SEPs). The SEPs responses from the upper limbs were analyzed with bipolar stimulation of the median nerve at the wrist. Because the clinical symptoms of RLS primarily occur in the lower extremities, it would be logical to examine the SEPs using posterior tibial nerve stimulation. However, we preferred to examine the SEPs from the upper

limbs for several reasons. Firstly, the median nerve stimulation provides a precise mapping [30], which is especially important during the patient's examination with tremor interfering with recording. Secondly, the analysis of SEPs parameters such as peak-latency (PL) and inter-peak intervals (IPI) allows us to neglect the height of patients. Thirdly, it is useful for the detection of subclinical manifestations in RLS from the upper limbs.

### Blink reflex

Another method for the processes of evaluating sensory and integrating sensory-motor in RLS for BR investigation. BR studies provide important information about the afferent and efferent pathways and are an excellent physiological tool for the functional integrity assessment of supraspinal structures and their descending influence on the brainstem [31, 32]. BR was assessed using a two-channel system. The supraorbital branches of the trigeminal nerves were stimulated with 20 mA electric impulses on each side. We investigated the onset latency (OL) and amplitude (A) of ipsilateral R1 response and bilateral R2 (ipsilateral and contralateral) responses.

### Sympathetic skin response

The SSR is a complex polysynaptic reflex, which courses through a common centrifugal path via sympathetic post-ganglionic unmyelinated fibers to sweat glands [33, 34]. The posterior hypothalamus is the "generator" of the response and implements the integration between somatic and autonomic nervous systems on various stimuli. Measurement of SSRs at the mid-palm of both hands following the electrical stimulation of the median nerve at the wrist. Attachment of active standard Ag/AG/Cl cup electrodes to the mid-palm of both hands and referenced to the palmar surface of the fourth finger.

### Holter monitoring

Heart rate through the effects of sympathetic and parasympathetic nervous systems is modulated, and analysis of changes in heart rate over time provides information about the autonomic function. In this connection, HM is a reliable, non-invasive method to study heart rate variability for investigating the interaction between the circadian system and autonomic function in PD patients with RLS. We applied 24-hour Holter electrocardiographic monitoring for the determination of the circadian index (CI). Using time-domain heart rate variability analysis was performed to detect sympathetic and parasympathetic influences on the cardiovascular system. Calculation of CI is the ratio of awaking (7 am to 10 pm) mean heart rate to sleep (11 pm to 6 am) mean heart rate.

### Neuroimaging

3T scanner (Discovery 750W, General Electric Healthcare, United States) MRI scanning was conducted on a 24-channel head coil. The MRI protocol included a T1weighted sequence for identifying structure and segmentation. Anatomic images were acquired in sagittal orientation with three-dimensional inversion recovery prepared fast spoiled gradient recalled sequence BRAVO TR 7.8 TE 3.2 (repetition time/echo time ratio = 1,900/2.2 ms, inversion time = 900 ms, flip angle = 9 degrees, matrix size = 256 × 256, slice thickness = 1.2 mm, voxel size = 1 × 1 × 1 $mm^3$, and sections = 176).

Neuroimaging data were processed using the FreeSurfer 6.0 software (https://surfer.nmr.mgh.harvard.edu/). All available parameters were calculated in automatic approach for volumetric measurements of brain structures succinctly like a segmentation of the subcortical white matter and deep gray matter structures, skull stripping, automated topology correction, and surface deformation along intensity gradients for optimal placement of the borders between gray matter, white matter, and cerebrospinal fluid, border tessellation between gray and white matter. Surfaces were visualized and inspected for errors by using Freeview GUI from FreeSurfer. PAG was segmented manually and then with using FreeSurfer we estimated the intensity of this segment.

75 patients with PD with age ranging from 40-70 years: 36 with RLS (RLS+) and 39-without RLS (RLS-) were examined. In the control group, 30 age-matching healthy individuals were included. Recruited patients were diagnosed as idiopathic PD according to the British Parkinson's disease Society Brain Bank criteria [35]. According to the Hoehn and Yahr scale, the severity of PD ranged from 1 to 3 stages. The study excluded patients if they have Deep Brain Stimulation implants or underwent and surgical procedures (pallidotomy, thalamotomy, etc.), cognitive impairment (less than 24 points on the Mini-Mental State Examination), diabetes mellitus, arterial hypertension, atrial fibrillation, frequent extrasystoles (more than 700 per day), atrioventricular block and used the drugs affecting the heart rhythm. The International RLS Study Group and International RLS Severity Scale were used for Diagnostic criteria. We also applied the Unified Parkinson's Disease Rating Scale (UPDRS) for the clinical evaluation of PD and Parkinson's disease Questionnaire (PDQ-39) for the assessment of the quality of life in PD patients [36].

By using Statistical 10.0 software statistical analysis was performed. The non-parametric Mann-Whitney U test was used to compare the differences between two independent groups and for dependent samples, Wilcoxon Matched Pairs Test was used. To measure the degree of association between two variables, we applied a Spearman rank correlation. A p-value < 0.05 was considered significant.

## Results

In table 1, a comparison of data between PD patients with and without RLS is given. Based on their data, there was no significant difference in age and the UPDRS scale. However, the disease in a group of patients with RLS was longer duration, and quality of life according to the PDQ-39 was significantly impaired ($p < 0.05$).

### SEPs study revealed

In the RLS+, the PL of N11, IPI N9–N13, N11–N13 was significantly shorter, and the IPI N13-N20 was longer as compared with the RLS- group.

**Table 1:** Comparison of PD patients with and without RLS.

| Parameters | RLS+ Me (Q1, Q3) (n = 36) | RLS- Me (Q1, Q3) (n = 39) |
|---|---|---|
| Age (years) | 59 (55; 65) | 57 (52; 64) |
| Gender ratio (males/females) | 15/21 | 17/22 |
| Duration of PD (years) | 8 (5; 13)* | 4 (3; 7) |
| UPDRS scale | 49 (40; 59) | 47 (41; 60) |
| PDQ-39 | 75 (56; 95)* | 44 (25; 63) |

Me (Q1, Q3) – Median (lower and upper quartiles).
* - Statistically significant difference ($p < 0.05$).

**Table 2:** Comparative evaluation of SEPs parameters.

| SEP Parameters | Side | RLS+ (n = 36) | RLS- (n = 39) | Control group (n = 30) |
|---|---|---|---|---|
| PL N20 (ms) | DSS | 20.6 (20.1; 21.4) | 20.45 (20; 20.9) | 19.4 (18.8; 19.9)** |
| | OS | 20.05 (19.7; 21.2) | 20 (19.2; 20.8) | 19.2 (18.5; 19.9)** |
| PL P 23 (ms) | DSS | 23.8 (23.1; 24.65) | 23.8 (23.5; 24) | 22.5 (21.8; 22.8)** |
| | OS | 23.6 (22.9; 24.5) | 23.5 (22.7; 24) | 22.4 (22; 22.7)** |
| PL N11 (ms) | DSS | 11.75 (11.05; 13)* | 12.6 (12; 13.1) | 11.9 (11.5; 12.5) |
| | OS | 12.2 (11.5; 13.4) | 12.3 (11.8; 13.1) | 11.9 (11.5; 12.6) |
| PL N13 (ms) | DSS | 13.7 (13; 14.55) | 14 (13.5; 14.6) | 13.85 (13.4; 14) |
| | OS | 13.9 (13.15; 14.8) | 14.1 (13.6; 14.7) | 13.6 (13.4; 13.8) |
| PL P8 (ms) | DSS | 8.1 (7.4; 8.5) | 8.46 (8.2; 8.64) | 8.45 (7.84; 8.84) |
| | OS | 7.93 (7.56; 8.57) | 8.7 (8.1; 9.34) | 8.3 (7.7; 8.7) |
| PL N9 (ms) | DSS | 9.9 (9.38; 10.75) | 10.3 (10.1; 10.85) | 9.8 (9.1; 10.6) |
| | OS | 10.1 (9.66; 10.7) | 10.3 (9.9; 11.1) | 9.7 (9.5; 10.9) |
| IPI N13-N20 (ms) | DSS | 6.95 (6.35; 7.5)* | 6.3 (5.7; 6.9) | 6.1 (5.8; 6.4) |
| | OS | 6.55 (6.05; 7.05)* | 6.27 (5.58; 6.61 ) | 6.25 (5.5; 7.3) |
| IPI N11-N13 (ms) | DSS | 1.65 (1.35; 2)* | 1.9 (1.66; 2) | 1.8 (1.7; 1.9) |
| | OS | 1.6 (1.3; 2 ) | 1.8(1.6; 2) | 1.5 (1.3; 1.8) |
| IPI N9-N20 (ms) | DSS | 10.1 (9.53; 10.43) | 10.3 (9.72; 10.7) | 9.7 (9; 10.5) |
| | OS | 10.1 (9.6; 10.8) | 9.9 (9.57; 10.7) | 9.75 (9; 10.4) |
| IPI N9-N13 (ms) | DSS | 3.78 (3.42; 3.99)* | 3.96 (3.8; 4.2 ) | 4.3 (4; 4.7) |
| | OS | 3.74 (3.4; 4.4) | 3.8 (3.4; 4.3) | 4 (3.7; 4.3) |
| A N20-P23 (mcV) | DSS | 4.43 (3.32; 5.03)* | 3.06 (2.4; 3.7 ) | 3.15 (2.4; 4.3)** |
| | OS | 4.12 (2.27; 4.87)* | 2.88 (1.72; 4.3) | 3.05 (1.95; 4.25) |
| A N13-P18 (mcV) | DSS | 3.08 (2.36; 3.5)* | 2.3 (1.3; 3.05) | 2.35 (1.84; 3.29) |
| | OS | 2.84 (2.36; 3.63)* | 2.13 (1.63; 2.67 ) | 2.22 (1.7; 3.15) |
| A P8-N9 (mcV) | DSS | 4.57 (3.51; 5.87)* | 3.5 (2.88; 4.6) | 3.45 (2.4; 5.7) |
| | OS | 3.87 (3.1; 5.21)* | 3.13 (1.81; 4.2) | 3.3 (2.8; 5.2) |

DSS - Dominant side of symptoms in PD; OS - Opposite side.
* - Statistically significant difference ($p < 0.05$) between 1st and 2nd groups.
** - Statistically significant difference ($p < 0.05$) between control and 2nd groups.

RLS+ observed a significant increase in the amplitudes N20-P23, N13-P18 and amplitude P8-N9 as compared to RLS-, which can reflect sensitization processes at the level of the spinal cord, brain stem, and thalamocortical projections in patients with RLS.

Comparison between the control and the RLS- group revealed that in PD patients without RLS, PL N20, P23 was significantly longer, and amplitude N20-P23 was lower than in healthy persons. Table 2 summarizes the SEPs findings.

## Investigation of the BR parameters

Observation of hyperexcitability trend responses of BR, which can reflect the insufficiency of the inhibitory mechanisms at the segmental level and deficiency of supraspinal descending control in PD patients with RLS. The hyperexcitable BR has considered if the amplitude of their second components were more than 0.5 mcV.

No significant differences were found between groups when comparing the OL and amplitude of the first BR component. In contrast, the OL of the second BR components (R2 ipsilateral and R2 contralateral) were shorter in PD patients with RLS in comparison to PD patients without RLS when stimulated DSS. The amplitudes of the second component in the RLS+ group were significantly higher than those in both the RLS- and the control groups (Table 3).

**Table 3:** Comparative evaluation of BR parameters.

| BR parameters | Side | RLS+ (n = 36) | RLS- (n = 39) | Control group (n = 30) |
|---|---|---|---|---|
| OL R1 ips (ms) | DSS | 11.2 (10.6; 12.2) | 11.6 (10.9; 12.2) | 11.4 (11.1; 11.8) |
| | OS | 11.2 (10.8; 12.1) | 11.6 (11; 12) | 11.3 (11.1; 11.9 |
| OL R2 ips (ms) | DSS | 33.7 (31.6; 36)* | 36.4 (32.6; 38) | 32.4 (30; 36) |
| | OS | 32 (30; 36.1) | 34.8 (31.3; 37.6) | 32.0 (30.1; 36.2 |
| OL R2 contr (ms) | DSS | 32 (30; 33.6)* | 36 (32; 38) | 32.5 (29; 36) |
| | OS | 31 (29; 36) | 34 (31; 37) | 32.4 (29. 35.8) |
| A R1 ips (mV) | DSS | 0.2 (0.1; 0.3) | 0.2 (0.1; 0.4) | 0.3 (0.2; 0.4) |
| | OS | 0.2 (0.1; 0.35) | 0.1 (0.1; 0.3) | 0.3 (0.2; 0.4) |
| A R2 ips (mV) | DSS | 0.6 (0.5; 0.8)*Δ | 0.2 (0.1; 0.3) | 0.3 (0.2; 0.6) |
| | OS | 0.3 (0.2; 0.5) | 0.2 (0.1; 0.4) | 0.3 (0.2; 0.5) |
| A R2 contr (mV) | DSS | 0.35 (0.2; 0.6)* | 0.2 (0.1; 0.3) | 0.2 (0.2; 0.4) |
| | OS | 0.2 (0.1; 0.45) | 0.2 (0.1; 0.3) | 0.2 (0.2; 0.5) |

DSS - Dominant side of symptoms in PD; OS - Opposite side.
* - Statistically significant difference ($p < 0.05$) between 1st and 2nd groups.
Δ - Statistically significant difference ($p < 0.05$) between control and 1st groups.

## Investigation of the SSR

SSR was mainly biphasic or rarely triphasic waves with different onset latency, amplitude, and shape of the responses [34]. Electrical stimuli application to the median nerve with an intensity of 5 – 20 mA. We used the amplitude and latency values of the response with the highest amplitude and shortest latency for evaluation. According to the shape and polarity of the waveform, the N-type of SSR with maximum negative deflection was recorded more frequently in PD patients without RLS (in 30 people (77.1%). In 6 patients (15.3%), the P-type revelation was with maximum positive deflection. The absence of SSR to the electrical stimulus with an intensity of 30 mA was observed in 3 patients (7.6%) and regarded as a sign of peripheral autonomic failure. Observation of P-type

recording in PD patients with RLS of 17 patients (47%) cases, are significantly more frequent than in the group of PD patients without RLS ($\chi^2$ = 7.73; p = 0.005).

Due to the variability of the SSR amplitudes, we conducted a comparative analysis of the SSR latency that was relatively stable and less dependent on the intensity of the electrical stimulus. In PD patients with RLS, the OL was significantly longer in comparison with PD patients without RLS (1.75 (1.6; 1.98) ms - in the 1st group and 1.5 (1.3; 1.63) ms – in the 2nd group, p = 0.0001). A strong positive correlation between the severity of RLS and latency was found in the 1st group (RSpearman = 0.71; p = 0.00001).

To evaluate the peculiarities of the daily variability SEP, BR, and SSR parameters, an examination of all patients was done twice at 8 am and 6 pm after 1-1.5 hours of levodopa administration regardless of the presence or absence of motor fluctuations. The table presents the results of the examination of patients in the morning. Analyzing the dynamics of SEP parameters, there was no observation of significant differences in latency and IPI, but we found an increase in amplitudes both on the dominant side of symptoms (DSS) and on the opposite side (OS) in the evening compared with morning time in PD patients with RLS. So amplitude N20-P23 increased from 4.43 (3.32; 5.03) to 4.84 (3.32; 5.03) mcV, (Wilcoxon Matched Pairs Test p = 0.0002) on DSS and from 4.12 (2.27; 4.87) to 4.61 (2.84; 4.95) mcV (p = 0.0008) on OS; amplitude N13-P18 – from 3.08 (2.36; 3.5) to 3.16 (2.62; 3.75) mcV (p = 0.03) on DSS and from 2.84 (1.36; 3.63) to 2.9 (2.44; 3.76) mcV (p = 0.003) on OS. At the same time, no differences were found in amplitude P8-N9 on both sides. Identified results may indicate enhanced sensitization processes in the evening at the level of the spinal cord, brain stem and thalamocortical projections in a group of PD patients with RLS. In contrast, in patients without RLS, no statistically significant differences were obtained in any of the amplitudes. The peculiarity of the control group was that the amplitudes decreased in the evening, for amplitude N20-P23 – from 3.15 (2.4; 4.3) to 3.02 (2.24; 4.25) mcV (p = 0.009) and for amplitude N13-P18 – from 2.35 (1.84; 3.29) to 2.18 (1.72; 3.2) mcV (p = 0.001). These results probably reflect the prevalence of general inhibition processes in the evening in healthy people.

Evaluating the dynamics of the BR parameters, in PD patients with RLS, a statistically significant increase in the amplitude of the second component was detected in the evening, at the same time the latencies of all components, as well as R1 amplitude, did not differ between periods. So the amplitude of the R2 ipsilateral component increased from 0.6 (0.5; 0.8) to 0.7 (0.5; 0.9) mV (p = 0.01) and R2 contralateral – from 0.35 (0.2; 0.6) to 0.5 (0.2; 0.8) mV (p = 0.002). The process enhancement of polysynaptic reflex indicates excitability by the evening in PD patients with RLS.

Prolongation of latency and decrease in amplitude of SSR is a general tendency in patients with PD. Based on the SSR amplitudes that have inter-individual as well as intra-individual variations and undergo rapid habituation with repeated stimulations. In our study, we first analyzed only the latency and the shape of the response. This allowed us to establish the P-type recording, according to the shape of the SSR, was observed more often in PD patients with RLS than in individuals without RLS. The P-type recording indicates the predominance of the SSR second phase which is associated with an increase in sweating during stimulation and reflects the activity of supraspinal (primarily hypothalamic) ergotropic centers. N-type recording points out a decrease in perspiration to stimulus. It reflects the activation of hypothalamic centers inhibiting sweating and is used to determine the level of trophotropic activity [33].

In the analysis of the daily SSR variability, the PD patients without RLS tended to produce low amplitudes for both the first phase and the second one, and in some cases, the P-wave was not recorded at all. Also, almost all patients without RLS did not detect changes in SSR type recording between morning and evening observations, also the change in the amplitude of responses were absent or minimal. Quite different results were obtained in the group of PD patients with RLS. Regardless of the SSR type recording initially they have highly variable responses during the day. This was mainly expressed in an increase in the amplitude of the P-wave by 1.5 - 2 times in 24 PD patients (66.6%) when researching at 6 pm. In the evening the decrease in the P-wave amplitude with the increase of the N-wave amplitude was recorded. In 7 PD patients (19.4%) and 5 people (13.8%), there was no change in SSR (amplitude and recording).

## Holter monitoring

Holter Monitoring we used to analyze the CI that provides information about the features of changes in heart rate during the day and night. The CI was significantly reduced in groups of patients with PD (1.21 (1.13; 1.38), compared with the control group (1.33 (1.23; 1.49), p = 0.001) but there was no difference between RLS+ group and RLS- group. But when analyzing the time-domain parameters of heart rate variability, a significant decrease in SDANN (Standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording) and an increase in pNN50 (number of pairs of adjacent NN intervals) values was observed in PD patients with RLS when compared with the patients without RLS. So, in RLS+ group the SDANN was 94 (66; 115) ms and pNN50 – 5 (1; 12); in RLS- group the SDANN was 108 (79; 136) ms and pNN50 – 2 (0; 60)% (P < 0.001).

## MRI studies with using voxel-based morphometry

In 31 PD patients with RLS and 33 PD patients without RLS, MRI studies were performed using voxel-based morphometry. A significant decrease in the grey matter volume of some structures of the brain in groups of PD patients regarded the presence or absence of RLS. According to our study, in patients with PD, marked dilatation was detected of the lateral ventricles on both sides. Moreover, the right and left caudate volume diminished as well as a reduced of the right and left precentral gyrus volume compared to the control group, which may reflect the general neurodegenerative process associated with PD. There were no significant differences in regional tissue density between control subjects and PD patients.

To clarify whether RLS in PD patients is associated with any specific morphological changes in gray matter, we evaluated brain morphometric differences between RLS+ and RLS- groups of PD patients. Both lateral ventricles and 4th-ventricle were markedly dilated and volumes in the postcentral gyrus and lingual gyrus from both sides were significantly lower in patients with RLS. It is noteworthy that in the group of patients with RLS there was a significant increase in the grey matter volume of the thalamus, anterior and posterior cingulate cortex compared with PD patients without RLS (Table 4).

**Table 4:** Brain regions showing significant differences in volume ($mm^3$) values between PD patients with RLS and without RLS.

| Brain regions | RLS+ (n = 31) | RLS- (n = 33) | P value |
|---|---|---|---|
| Left Lateral Ventricle | 11772 (11684; 15452) | 10255 (6746; 13307) | p = 0.055 |
| Right Lateral Ventricle | 12647 (10733; 14882) | 9789 (6316; 12473) | p = 0.016 |
| 4th Ventricle | 2197 (1503; 2873) | 1717 (1384; 2074) | p = 0.002 |
| Left Thalamus | 7847 (6896; 8943) | 7623 (6597; 7847) | P = 0.043 |
| Right Thalamus | 7834 (6813; 8281) | 7042 (6275; 7834) | p = 0.026 |
| Left Postcentral Gyrus | 7680 (7174; 8674) | 8278 (7591; 9739) | p = 0.037 |
| Right Postcentral Gyrus | 7365 (6942; 8894) | 8300 (7533; 9020) | p = 0.037 |
| Posterior Cingulate Cortex | 1082 (954; 1130) | 919 (852; 960) | p = 0.002 |
| Anterior Cingulate Cortex | 980 (859.4; 1076.4) | 859.3 (745; 980) | p = 0.027 |
| Left Lingual Gyrus | 5625 (4866; 5750) | 5948 (5474; 6194) | P = 0.017 |
| Right Lingual Gyrus | 5792 (5061; 6083) | 6249 (5940; 6883) | p = 0.006 |

The periaqueductal gray matter (PAG) is a poorly differentiated midbrain structure and attempts to segment the human PAG have unreliable results due to its small size and location within the brainstem surrounding the cerebral aqueduct. Undoubtedly, the altered PAG function [17, 18] may result in the change of PAG volume, but due to certain difficulties in determining the exact boundaries of this structure, we conducted a comparative analysis of the MRI signal intensity only within identified areas of the PAG in groups of examined patients. The following signal intensity values were detected: for the control group – 67.55 (65.69; 70.06), for the RLS+ group – 71.13 (67.18; 71.87) and RLS- group – 61.30 (58.05; 64.14). Comparative analysis revealed no statistically significant differences between PD patients with RLS and the control group (p = 0.091). Meanwhile, in patients with RLS, the MRI signal intensity was significantly higher than in PD patients without RLS (p = 0.00045).

## Discussion

An increase in the IPI N13-N20 of SEPs indicates a deceleration of the signal transmission along with the thalamocortical projection in PD patients as compared to healthy people, which can reflect neurophysiological features characteristic of PD in general. We observed that patients with RLS had more prolonged IPI N13-N20, but at the same time a significant shortening of the IPI N9–N13, N11–N13 was revealed as compared with PD patients without RLS. Due to changing the sensory response elicited by generating an augmented action potential output, this finding, as well as an increase in amplitudes N20-P23, N13-P18, and P8-N9, maybe a reflection of neuronal hypersensitivity at the level of the spinal cord, brainstem and thalamocortical projections.

A decrease in latency and an increase in the amplitude of second BR components indicate an increase in polysynaptic reflex excitability in PD patients with RLS. The tendency to hyperexcitable BR responses is explained by the insufficiency of inhibition mechanisms at the level of the brainstem, spinal cord segment, and deficiency of supraspinal descending control in PD patients with RLS. This is probably one of the fundamental factors predisposing to voluntary and involuntary movements in the legs.

The SSR provided the assessment of sympathetic fibers impairment as well as disorders of supraspinal structures of the brain participating in autonomic regulation of the sweat glands function. We revealed a significant increase in the SSR latency in PD patients with RLS. It can reflect the synaptic delay duration at the level of the brain and superior cervical ganglion. Although the central parts of the SSR reflex arc are known incompletely, the mesencephalic reticular formation, posterior hypothalamus, caudate nucleus, and others are the most important neural structures generating the reflex [34]. The positive correlation between the severity of RLS and SSR latency indicates that the degree of RLS depends on the severity of functional disorders in the supraspinal autonomic centers. In PD patients with RLS, P waveforms type recording was observed more often, which can point out the predominance of sympathetic influence on the SSR. It is known that A11 dopaminergic modulatory action on sympathetic preganglionic neurons in the IML is predominantly inhibitory and its absence would favor increased basal sympathetic tone [11, 37-39]. But in fact, most PD patients without RLS tend to give the opposite N-type recording which indicated the predominance of parasympathetic influence on the SSR. Descending pathways from the A11 region also project to the dorsal raphe nucleus [40]. The serotonergic dorsal raphe promotes cardiovascular and sympathetic activity, and it has, like the dopaminergic A11, prominent projections to the IML [41]. In contrast to dopamine, its actions are strongly excitatory [11]. Insufficiency in this system leading to inhibitory effects on preganglionic sympathetic neurons can explain the predominance of parasympathetic activity in PD. In the case of RLS, there is probably a tendency to sympathetic activity due to the predominance of exciting supraspinal influence on the intermediolateral nuclei of the spinal cord.

Whereas RLS has significant circadian pattern which presents as worsening symptoms in the evening and remission in the morning after waking up [42], it was important to

identify of the N20-P23 and N13-P18 amplitudes increasing of the SEP, as well as increasing of the R2 ipsilateral and contralateral BR amplitudes in the evening in PD patients with RLS. In other words, significant enhancement of excitation processes at the spinal cord, brainstem, and thalamocortical level are the basic neurophysiological mechanisms reflecting the circadian pattern of clinical manifestations of RLS.

When assessing the daily variability of the SSR, it should be noted that its parameters are not stable in healthy people and dependent on many internal and external factors, such as temperature, psycho-emotional state, medication, etc. However, PD patients without RLS had almost no variability of reflex parameters during the day. The absence of significant changes in SSR between morning and evening observations, on the one hand, maybe explained by denervation of the peripheral sympathetic nerves innervating the sweat glands, but on the other hand, reflect a disturbance in the centers of supraspinal autonomic system, their peculiar "rigidity" to give an adequate response to stimulation. In PD patients with RLS, the ability to daily variation of SSR parameters was noted, while in most cases, there was a tendency to an increase in sympathetic influences on the sweat glands in the evening.

The change in CI indicates a disorder of the correct ratio of heart rate between night and day time, which may also point to the insufficiency of the mechanisms that regulate circadian processes in general. Moreover, the effects of sympathetic and parasympathetic nervous systems modulate the heart rate, analysis of changes in heart rate over time provides useful information about the autonomic function. According to our research, a decrease in the CI, as it turned out, is the general trend of all patients with PD. At the same time, the decrease of SDANN and increases in pNN50 values were typical for PD patients with RLS. Together these findings suggest autonomic dysfunction in the form of increased sympathetic activity.

Disturbance of somatosensory processes and autonomic regulation in RLS suggests the presence of certain morphological changes in various areas of the brain. Voxel-based morphometry offers the opportunity to investigate subtle changes in gray matter volume [43, 44]. The revealed dilatation of both lateral ventricles in PD patients compared with the control group can be regarded as a non-specific process associated with brain atrophy. At the same time, decrease grey matter atrophy observed in both caudate and precentral gyrus may give the evidence of structural alteration due to degeneration related to PD. In previous papers, researchers also reported about volume reduction in the putamen, caudate, hippocampus, and other structures of the brain which are an essential part of neural networks involved in motor and nonmotor function [45-47]. Nevertheless, the involvement of certain structures in PD degeneration varies in different studies, and therefore this question remains controversial. The differences in the results probably explained by the following reasons: 1) using various MRI techniques that can detect changes in the volume and density of the brain structures; 2) the uneven involvement of various cortico-basal ganglia-thalamocortical loops determining the difference in the clinical picture in each case; 3) the presence of certain clinical symptoms due to changes in cerebral function do not always parallel changes in structure.

In connection with the above statement, the identification of any morphological change in gray matter characteristic for RLS in PD patients presents an even more challenging task. To reveal specific brain structural alterations we tried to find differences in the voxel-based morphometry results between PD patients with presence and absence RLS. According to our research, persons with RLS had a more pronounced dilatation of not only the lateral ventricles but also IV ventricles, which may indirectly indicate the presence of a more significant atrophic process in the medulla oblongata and the pons compared with PD patients without RLS. Logical to assume that decreases of gray matter volume in the postcentral gyrus are a consequence of impaired somatosensory processes associated with the RLS, but what was interesting was that the volume of the thalamus in this category of patients was larger. Another unexpected result was a significant increase of posterior and anterior cingulate cortex volume in PD with RLS. Among the various important functions, these structures perform a prominent role in pain and episodic memory retrieval as well as in a wide variety of autonomic functions, such as regulating blood pressure and heart rate [48, 49]. Probably the increase in the volume of posterior and anterior cingulate cortex can act as an anatomic substrate of autonomic dysfunction and overactive somatosensory processes due to pathological impulses from spinal and brainstem levels revealed by our neurophysiological research methods in PD patients with RLS.

One of the key brain structures that can be closely related to the pathogenesis of the RLS is PAG. Most of these targets of PAG inputs are premotor centers that in turn project to sensory, motor, or autonomic nuclei of the brainstem and spinal cord [50]. PAG network also includes the prefrontal and anterior cingulate cortex, hypothalamus, amygdala, dorsolateral pontine reticular formation, rostral ventromedial medulla, and caudal rostral ventromedial medulla [50, 51]. Through connections with these structures, the PAG coordinates specific patterns of cardiovascular, respiratory, motor, and pain modulatory responses. It was established that PAG participates in control behavior-specific patterns of motor and autonomic responses and modulate both dorsal horn, trigeminal nucleus excitability to nociceptive inputs and gain of spinal reflexes [50]. In experimental studies, it was found that dorsal PAG stimulation may elicit increases in sympathetic activity and baroreflex sensitivity [52]. Stimulation of the ventrolateral and ventral PAG elicited a change in the balance between vagal and sympathetic modulation of the heart rate and induced analgesia [53, 54].

Detected of a statistically significant increase in the intensity of PAG in persons with RLS along with the presence of other PD-related morphological changes in the brain may indirectly indicate its dysfunction. This idea can be supported by our neurophysiological data that specify the presence of polysynaptic reflex excitability and sensitization processes at the different level of the somatosensory system as well as the predominance of sympathetic influence on the SSR and heart rate especially at night in PD patients with RLS. The enhancement of excitation processes in the somatosensory

system in the evening realized through PAG, namely through disturbances in the influence of PAG-projecting RGCs on the activity of PAG neurons that eventually also contribute to the disorders in the circadian regulation of sensory, motor, and autonomic functions.

Thus, in our study, we have attempted to determine the main neurophysiological components of the RLS with the assessment of their variability during the day and also find their characteristic structural changes in the brain. The complexity of the RLS pathogenesis requires further more detailed studies that were not mentioned in this article. In our opinion, to expand the understanding of the RLS in PD patients, it would be appropriate to present neuropsychological assessment in relationship with those morphological changes in the brain. Using the MRI 3T scanner did not determine changes in the PAG volume, and in LC volume, which is also important in the pathogenesis of the RLS. In this regard, the use of more powerful MRI devices in our next studies will allow us to resolve this issue.

## Conclusion

Revealed disorders in the sensory, motor and autonomic systems expressed at different levels in PD patients with RLS in the functional state changes of the subcortical-brainstem-spinal systems. Disturbance of somatosensory processes and autonomic regulation have daily variability which reflects the circadian pattern of RLS in PD. Identified through voxel-based analysis of MTR images, more pronounced dilatation of the lateral and IV ventricles indicates the more significant non-specific brain atrophy process in PD patients with RLS. On this background, the increase of the thalamus volume, posterior and anterior cingulate cortex volume, as well as the amplification of the PAG signal intensity in this category of patients, can have a direct relation to autonomic dysfunction leading to change of cardiovascular, motor patterns, and overactive somatosensory processes.

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## Conflict of Interest

The authors declare no conflict of interest

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