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Original Article

Short-interval intracortical inhibition is decreased in restless legs syndrome across a range of severity

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ABSTRACT

Background: Decreased short-interval intracortical inhibition (SICI) to transcranial magnetic stimulation (TMS) of the primary motor cortex was described in subjects with restless legs syndrome/Willis-Ekbom disease (RLS/WED). It remained to be determined whether the magnitude of SICI decrease would be similar across levels of RLS/WED severity. Moreover, it was unknown whether, in addition to decreases in SICI, changes in cortical thickness or area could be detected in subjects with RLS/WED compared to controls. The objective of this study was to compare SICI, cortical thickness, and cortical area in subjects with idiopathic mild to moderate RLS/WED, severe to very severe RLS/WED, and controls.

Methods: The severity of RLS/WED was assessed by the International Restless Legs Syndrome Severity Scale (IRLSS). SICI and 3T magnetic resonance imaging (MRI) data of subjects with RLS/WED and controls were compared. A receiver operating characteristic curve for SICI was designed for discrimination of participants with RLS/WED from controls. Cortical thickness and area were assessed by automated surface-based analysis.

Results: SICI was significantly reduced in patients with mild to moderate and severe to very severe RLS/WED, compared to controls (one-way analysis of variance: $F = 9.62$, $p < 0.001$). Receiver operating characteristic curve analysis predicted RLS/WED when SICI was above 35% (area under the curve = 0.79, 95% CI 0.67–0.91, $p < 0.001$). Analyses of the whole brain and of regions of interest did not reveal differences in gray matter thickness or area between controls and subjects with RLS/WED.

Conclusion: SICI is an accurate cortical biomarker that can support the diagnosis of RLS/WED even in subjects with mild symptoms, but cortical thickness and area were not useful for discriminating subjects with this condition from controls.

1. Introduction

Restless legs syndrome (RLS), also known as Willis-Ekbom disease (WED), affects 1–11.6% of the general population [1]. It is characterized by an urge to move the limbs, usually accompanied by discomfort or unpleasant sensations, that predominantly occur

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in the evening and are partially relieved by moving the affected body part [2,3].

The diagnosis is based on clinical evaluation. Two distinct forms of RLS/WED are described. In idiopathic RLS/WED (which is not associated with other conditions) there is usually a genetic inheritance [4], and neurological examination results are normal [5]. The exact mechanisms underlying this condition remain unknown. It has been hypothesized that abnormal dopaminergic activity in the striatum might lead to abnormal excitability of the motor cortex [6,7], or that defective cortical inhibition itself might underlie RLS/WED pathogenesis. From positron emission tomography (PET) studies, there is some evidence of decreased pre- [8,9] and post-synaptic [9–11] dopamine uptake in the striatum and of decreased activity in mesolimbic areas [12]. However, the absence of abnormalities in striatal dopaminergic activity has also been reported [13–15].

Transcranial magnetic stimulation (TMS) is a valuable tool to examine cortical excitability. Although there is controversy about excitability to TMS in RLS/WED (reviewed elsewhere [16,17]), most studies have shown decreased short-interval intracortical inhibition (SICI) in the primary motor cortex [6,7,18–23]. It has been suggested that lower than normal GABA-A activity in cortical interneurons may result in decreased SICI [24,25].

It remains to be determined whether the magnitude of SICI decrease would be similar across levels of RLS/WED severity. This question was examined in the present study by contrasting the dominant motor cortex SICI in participants classified by the International Restless Legs Syndrome Group Scale (IRLSS) as severe/very severe (RLS/WED$_{SVS}$ subgroup, IRLSS $\geq$20), or mild/moderate (RLS/WED$_{MM}$ subgroup, IRLSS <20) and controls. We hypothesized that SICI would be reduced in the RLS/WED$_{SVS}$, but not in the RLS/WED$_{MM}$ subgroup, compared to the control group. As a secondary goal, we evaluated correlations between SICI and severity of RLS/WED symptoms. We also assessed sensitivity and specificity of SICI for RLS/WED diagnosis.

Decreased SICI could reflect abnormal motor cortical processing that may be primary or secondary to subtle structural changes in other regions of the brain [26,27]. Magnetic resonance imaging (MRI) studies in RLS/WED have reported variable results regarding gray matter morphometry: increases in the thalamic pulvinar [28]; decreases in the primary somatosensory [29] and primary motor cortices [30]; decreases in the cerebellum [31]; increases [32] or decreases in the hippocampus and other brain regions [31], or no changes [33–38]. Except for two studies [29,32], none of these studies included corrections for multiple comparisons in their statistical analyses. In addition, heterogeneous sample sizes and clinical characteristics of the patients may have concurred to the heterogeneous findings.

With few exceptions [29,31,34], most studies collected MRI data with low spatial resolution acquisitions and performed voxel-based morphometry (VBM) analyses [39]. Surface-based analysis (SBA) is an alternative procedure to evaluate changes in gray matter, especially for the estimation of its cortical thickness. FreeSurfer, a full processing pipeline for structural MRI data, is especially designed for SBA [40,41], which improves matching of homologous cortical regions within and between subjects, allowing more accurate detection of cortical borders based on probabilistic atlases [42,43]. A further advantage of SBA over VBM analysis methods is the substantive reduction of residual inter-subject anatomical variability [44–47]. A recent study applying SBA found modest reduction of cortical thickness in somatosensory cortices in patients with RLS/WED when compared to controls [29]. In the present study, we investigate SBA with high-resolution T1 in individuals with RLS/WED. Gray matter structure in controls and RLS/WED were examined through whole-brain analysis. We also assessed regions of interest (ROI) previously shown to be involved in RLS/WED pathogenesis (primary somatosensory cortex, primary motor cortex, caudate nucleus, pallidum, putamen and thalamus) [28–32,34]. Based on previous findings, we expected to find decreased gray matter thickness or area in primary motor and somatosensory cortices, as well as decreased volumes of subcortical regions in the RLS/WED group [28–32,34].

2. Methods

2.1. Subjects

Subjects with idiopathic RLS/WED were recruited at a specialized sleep center (Neuro-sono, UNIFESP) and in the community of São Paulo, Brazil, from November 2013 until August 2015. Age- and sex-matched controls were recruited among hospital workers, acquaintances from the researchers, and nonrelative contacts of subjects with RLS/WED.

A medical interview and clinical and neurological examination were performed by a board-certified sleep medicine specialist (S.C.M.) in all subjects. We assessed age, sex, ethnic group, and handedness measured by the Edinburgh Inventory [48]. For subjects with RLS/WED, disease duration, symptomatic body region, family history, treatments, severity, and quality of life were also evaluated. Cognitive, mood, and clinical status were nonstructured assessed during medical interview. Because SICI may be influenced by hormonal fluctuation [49], women were asked about their menopausal status and menstrual cycle. Women of childbearing age were classified according to current use of oral contraceptives, luteal phase, or follicular phase. The severity of RLS/WED symptoms was measured by the IRLSS [50,51] and the quality of life by the Restless Legs Syndrome Quality of Life Questionnaire [52]. Excessive daytime sleepiness was assessed by the Epworth Sleepiness Scale [53,54]. These scales were administered on the day of the TMS study by a researcher not involved in TMS testing (J.P.).

Inclusion criteria for patients were age 18–70 years, with idiopathic RLS/WED diagnosed according to the criteria of the International RLS Study Group (IRLSSG) [2,55] and normal brain MRI findings. Subjects were excluded from the study if they had any history of other neurological or psychiatric diseases, family history of RLS/WED, or clinical findings consistent with secondary RLS/WED [3,55].

For controls, inclusion criteria were age 18–70 years, and sex and handedness similar to those of patients. Exclusion criteria for controls were personal or family history of RLS/WED symptoms and history of neurological or psychiatric comorbidities.

In addition, exclusion criteria for both subjects with RLS/WED and controls were contraindications to TMS [56], use of medications that could influence excitability to TMS, such as opioids, sedative hypnotics, antipsychotics, antidepressants, β-blockers, stimulants, mood stabilizers, and drugs for RLS/WED treatment within the past two weeks.

The local ethics committee approved the experimental protocol, and all subjects provided written informed consent. All procedures were performed in accordance with the ethical standards of the institutional and national research committees, as well as with the 1964 Declaration of Helsinki and its later amendments.

2.2. Experimental design

For subjects receiving treatment for RLS/WED, a minimum washout period of one week was required prior to TMS testing. TMS

and MRI were performed on the same day to enhance feasibility of the protocol and to limit washout duration to a minimum.

2.3. Outcomes

The primary outcome was SICI in the motor cortex of the dominant hemisphere. Secondary outcomes were as follows: correlations between SICI and IRLSS scores in subjects with RLS/WED; diagnostic accuracy of SICI to discriminate RLS/WED and controls; cortical gray matter thickness and surface area in the primary motor and somatosensory cortices; volumes of the caudate nucleus, pallidum, putamen, and thalamus; as well as voxel-by-voxel gray matter assessed with a whole-brain, exploratory approach. The researcher who administered TMS was not blinded to RLS/WED or control status but was blinded to IRLSS scores (assessed by a research assistant). Additionally, SICI was blindly analyzed.

2.4. TMS

TMS was performed as described elsewhere [57]. TMS testing was performed exclusively in the afternoon, between 14:00 and 17:00. All volunteers were instructed not to ingest alcohol (48 h) and caffeine (24 h) before TMS testing [58,59]. We administered monophasic pulses to the dominant primary motor area with a figure-of-eight coil (MC-B70, 2 × 97 mm, 31 kT/s) connected to a Magpro X100 stimulator (MagVenture, Farum, Denmark). For evaluation of SICI, the intensity of the conditioning pulse was kept at 80% of the resting motor threshold (rMT), classically proposed as the conditioning stimulus that leads to robust suppression of the test stimulus response in the paired-pulse paradigm [24]. Conditioning stimulus in SICI paradigm has the role of stimulating inhibitory interneurons that diminish descending volleys elicited by test stimuli. The definition of which intensity would highly reduce the motor evoked potential (MEP) amplitude induced by test stimulus has been discussed by many authors [60,61]. There is an indication that a conditioning stimulus of 60–80% resting motor threshold (rMT), as used in our protocol, would employ the greatest GABAergic inhibition over the test stimulus [24,62]. Test stimuli were adjusted to elicit MEPs with amplitudes between 0.5 and 1 mV (peak-to-peak), typically at intensities around 130% rMT [24].

The interstimulus interval was 2 ms. A total of 10 conditioned trials were recorded randomly, intermingled with 10 test trials, with intervals varying between 5 and 7 s. The order of the conditioned and test trials was randomized. In each trial, 100 ms (500 datapoints) were registered post-TMS pulses, and 40 ms (200 datapoints) pre-TMS pulses. The conditional triggering feature was used to deliver TMS stimuli only when the abductor digiti minimi (ADM) muscle was relaxed (electromyographic [EMG] activity at baseline $\leq$50 μV, for at least 1 s) [63].

The raw data were visually inspected (J.P.) and blindly analyzed by another investigator (S.C.M.). Trials with artifacts were discarded from the analysis. MEP amplitudes (peak-to-peak) were measured using the playback feature of LabVIEW [63]. Mean amplitudes of the conditioned and test MEPs were then calculated. Outliers (±2 standard deviations of the MEPs' mean value) were excluded. At least 5 conditioned and 5 test trials of appropriate quality were required for inclusion of the data in the analysis. SICI was expressed as the relative change (%) in mean MEP amplitude of the conditioned MEPs, in relation to the test MEPs [24,64]. Greater values reflect lower SICI.

Before and after the TMS experiment, leg discomfort was evaluated with a visual analogue scale (VAS) [65,66] The presence of discomfort was classified as absent (VAS = 0) or present (VAS >0).

2.5. Magnetic resonance image acquisition and processing

MRI was performed on a 3.0 T MR system equipped with 45 mT/m strength, 200 T/m/s slew rate gradient system and a 32-channel head coil (MAGNETOM Tim-Trio, Siemens Healthineers, Erlangen, Germany). High-resolution T1-weighted images were acquired using a 3D magnetization-prepared rapid-acquisition gradient echo (MPRAGE) acquisition: 176 slices, 0.50 × 0.50 × 0.99 mm^3 isotropic voxels, matrix 256 × 256, field of view 256 × 256, flip angle 7°, TR 2500 ms, TE 3.45 ms) [67]. MRIs were reviewed by a board-certified, experienced neuroradiologist (E.A.Jr.) to exclude relevant structural abnormalities. White matter T2-weighted hyperintensities and mild visually detected atrophy were allowed.

SBA was performed with FreeSurfer suite version 6.0.0 (http://surfer.nmr.mgh.harvard.edu). Details and technical aspects of this processing stream have been described elsewhere [68]. Briefly, T1-weighted images were analyzed in a processing pipeline, which includes brain extraction, Talairach transformation, segmentation of cortical and subcortical structures, intensity normalization, gray matter (GM) -white matter (WM) boundary tessellation to produce a surface, which is then corrected to ensure the same topology of a sphere [69,70]. We also adopted the recon-all flag "-3T" that uses more appropriate parameters for 3T MRI [71,72], as well as a 3T-based atlas for Talairach alignment [73] to improve its accuracy [74]. The process resulted in the reconstruction of the GM and WM surfaces, providing limits for GM thickness calculation as the minimal orthogonal distance between corresponding triangles in the two surfaces. Automated parcellation divided cortical surface into anatomical structures, based on the Desikan–Killiany Atlas [75], which labels the primary motor cortex as "pre-central area" and the primary somatosensory cortex as "post–central area." Cytoarchitectural probability maps defining Brodmann areas of interest (BA 1, 2, 3a, 3b, 4a, 4p) data were also overlaid [42]. Subcortical volumes and total intracranial volume were also calculated with FreeSurfer [76].

2.6. Data analysis

2.6.1. Characteristics of the subjects

Categorical variables were described as frequencies. Continuous variables were expressed as means and standard deviations, or as medians and interquartile ranges. Demographic and clinical variables between RLS/WED_{SVS}, RLS/WED_{MM}, and those of the control group were compared with one-way analysis of variance (ANOVA), the Kruskal–Wallis test, or the Fisher exact test according to data type and distribution. For comparisons among RLS/WED_{MM} and RLS/WED_{SVS}, we used the Student *t*, Mann–Whitney, or Fisher exact test.

2.6.2. TMS outcomes

TMS outcomes were compared between the RLS/WED_{MM}, RLS/WED_{SVS}, and those of control groups with one-way ANOVA and Tukey post hoc tests, when applicable. SICI results were not normally distributed and hence were log-transformed. The correlation between SICI and IRLSS was assessed with the Kendall Tau-b correlation coefficient. *P* Values $\leq$ 0.05 were considered statistically significant.

We used the Student *t* test to compare log-transformed SICI values obtained from subjects with RLS/WED who reported leg discomfort during the TMS experiment and those who did not.

A receiver operating characteristic (ROC) curve was built to evaluate sensitivity, specificity and accuracy of SICI to discriminate participants with RLS/WED from controls [77]. Accuracy was based on the area under the curve (AUC). AUC >0.70 was considered relevant [77,78]. The cut-off threshold that yielded the highest

combined sensitivity and specificity for distinguishing RLS/WED subjects from controls was identified by the Youden index [79].

2.6.3. Surface-based morphometric analysis

Vertexwise morphometric analysis of cortical thickness and surface area were performed using the Query, Design, Estimate, Contrast (QDEC) application of FreeSurfer, after smoothing both with Gaussian kernels with full width at half maximum (FWHM) of (a) 10 and (b) 20 mm. The design included age and sex as nuisance variables and accommodated the possibility of interaction group by age and group by sex Different Onset, Different Slopes (DODS). Correction for multiple testing was done with the false discovery rate (FDR) set at 5%. In addition, an ROI-based approach was performed for the primary motor cortex and primary somatosensory cortex (cortical thickness and area), as well as for the caudate, putamen, pallidum, and thalamus (volume), using SPSS (version 22.0), with a general linear model (GLM) that, likewise, included age and sex as nuisance variables. When we compared the volume of subcortical structures between RLS/WED and controls, the GLM also included total intracranial volume to accommodate individual differences in head size [80].

We performed a whole-brain analysis because some studies [30–32] reported scattered structural abnormalities in RLS/WED subjects not directly related to motor and somatosensory functions. Power analysis was performed with the FSpwr tool [81], considered the FWHM of 10 mm for the vertexwise models, as it is more conservative than for 20 mm.

3. Results

3.1. Characteristics of the subjects

A total of 284 subjects were screened. A flowchart of subject recruitment through the study is provided in Supplementary Fig. 1.

TMS and MRI data from 39 subjects with idiopathic RLS/WED and 28 controls were collected. From the RLS/WED group, we excluded the following: three subjects because of artifacts in TMS recordings; two subjects who did not present MEPs at the maximum stimulator's output; two subjects with incidental lesions on MRI (one with lacunar infarct in the pons, another with right cerebellar ischemia and Chiari 1 malformation); and one subject with low-quality MR acquisition due to excess of motion. We could not obtain MEPs in one control and excluded TMS data from two other subjects due to artifacts. In addition, two controls discovered, after joining the study, first-degree relatives with personal histories of RLS/WED symptoms and were excluded. Hence, we analyzed data from 31 patients with RLS/WED (26 drug-naive) and 23 controls. Demographic and clinical data are shown in Tables 1 and 2.

There were no significant differences in age, sex, handedness or ethnicity between the RLS/WED and control groups. Except for two participants who used levothyroxine due to (clinically compensated) hypothyroidism, none were on opioids, sedative hypnotics, antipsychotics, antidepressants, β-blockers, stimulants, mood stabilizers, or medications for diabetes [82].

There was a trend for higher Epworth Sleepiness Scale scores in the RLS/WED_{SVS} subgroup compared to the RLS/WED_{MM} subgroup. The frequency of symptoms was higher, disease duration was longer, and quality of life was worse in the RLS/WED_{SVS} than in the RLS/WED_{MM} subgroup (Table 1). Menstrual cycle phase and oral contraceptive use in women of childbearing age were not different between groups (Pearson χ^2: 3.38; $p = 0.18$).

3.2. TMS outcomes

3.2.1. Primary outcome: SICI

SICI was significantly reduced in RLS/WED_{SVS} (mean ± SD = 55.1% ± 40.9%) and RLS/WED_{MM} (59.6% ± 41.0%) subgroups, compared to controls (23.1% ± 14.2%) (one-way ANOVA: $F = 9.62$, $p < 0.001$, effect size: 0.277). Post hoc analysis showed significantly shorter SICI in the RLS/WED_{SVS} subgroup in comparison to the control group ($p = 0.002$), as well as between the RLS/WED_{MM} subgroup and the control group ($p = 0.002$), but not between RLS/WED_{SVS} and RLS/WED_{MM} subgroups (Fig. 1A). There was no significant correlation between SICI and IRLSS scores (Kendall Tau b = 0.0164, $p = 0.913$) (Fig. 1B).

When we excluded five patients with history of drug treatment for RLS/WED from the analysis, the above-mentioned significant differences remained.

Notably, there was a trend ($p = 0.08$) for decreased SICI ($n = 12$, 70.8% ± 42.9%) in patients who reported leg discomfort according to the visual analogue scale before or after TMS testing, compared to those who did not ($n = 19$; 50.1% ± 37.3%). The presence of symptoms before or after the TMS experiment was not different between the RLS/WED_{MM} and RLS/WED_{SVS} subgroups ($p = 0.332$).

3.2.2. Secondary outcomes: sensitivity and specificity of SICI for RLS/WED diagnosis

The ROC curve analysis predicted RLS/WED when the SICI cutoff was above 35.0% with a sensitivity of 66.7% and specificity of 86.9% (AUC = 0.79, 95% confidence interval [CI] = 0.67–0.91, $p < 0.001$) (Fig. 1C) [77].

3.3. Surface-based morphometric analysis

QDEC whole-brain analysis did not show any relevant cluster when data from controls and RLS/WED subjects were compared.

Table 1
Characteristics of control subjects and subjects with idiopathic restless legs syndrome/Willis-Ekbom disease (RLS/WED) by subgroups.

Characteristics	Control ($n = 23$)	RLS/WED mild to moderate ($n = 12$)	RLS/WED severe to very severe ($n = 19$)	p
Age, y, mean (SD)	41.7 (11.2)	42.7 (15.9)	47.6 (13.2)	0.32[a]
Gender, female, n (%)	21 (91)	10 (83.3)	15 (78.9)	0.48[b]
Ethnic group, n (%)				0.54[b]
White	20 (87.0)	12 (100.0)	16 (84.2)	
Asian	0 (0.0)	0 (0.0)	1 (5.3)	
Black	3 (13.0)	0 (0.0)	2 (10.5)	
Handedness, right-handed, n (%)	22 (95.7)	12 (100.0)	18 (94.7)	1.00[b]
Oldfield inventory, median (IQR)	83.3 (33.3)	79.1 (20.8)	83.3 (16.6)	0.96[c]
Epworth Sleepiness Scale, mean (SD)	8.1 (3.6)	10.7 (5.4)	11.3 (5.1)	0.07[a]

IQR, interquartile range; SD, standard deviation.
[a] p Value: one-way analysis of variance.
[b] p Value: Fisher exact test.
[c] p Value: Kruskal–Wallis test.

Table 2
Clinical characteristics of subjects with idiopathic restless legs syndrome/Willis-Ekbom disease (RLS/WED) with mild to moderate or severe to very severe symptoms.

	RLS/WED mild to moderate (n = 12)	RLS/WED severe to very severe (n = 19)	*p*
Age of onset (years) – Median (IQR)	27.5 (27.0)	20.0 (22.0)	0.26[a]
Disease progression (years) – Median (IQR)	6.5 (10.7)	24.0 (22.0)	**0.01**[a]
Positive RLS/WED family history – n (%)	10 (83.3)	12 (63.2)	0.41[b]
RLS/WED symptoms' frequency			**<0.001**[b]
≥1x/year < 1x/month	2 (16.7)	0 (0.0)	
≥1x/month < 1x/week	7 (58.3)	1 (5.3)	
≥1x/week	3 (25.0)	18 (94.7)	
Symptoms present in more than one region (in addition to the legs) – n (%)	2 (16.7)	5 (26.3)	0.67[b]
Previous RLS/WED treatment – n (%)	0 (0.0)	5 (26.3)	0.12[c]
IRLSS – n (%)			NT
mild (0–10)	4 (33.3)	0 (0.0)	
moderate (11–19)	8 (66.7)	0 (0.0)	
severe (20–30)	0 (0.0)	12 (57.1)	
very severe (31–40)	0 (0.0)	7 (33.3)	
RLSQoL scale score (%) – Mean (SD)	77.6 (15.4)	56.5 (14.3)	**<0.001**[c]

IQR, interquartile range; NT, not tested; RLSQoL, Restless Legs Syndrome Quality of Life Questionnaire; SD, standard deviation.
[a] p Value: Mann–Whitney test.
[b] p Value: Fisher exact test.
[c] p Value: Student t test.

Post hoc power analysis with the FSpwr [81] tool revealed a statistical power of 83.6% to detect a significant difference in cortical gray matter thickness with a 10-mm smoothing FWHM.

No significant differences in cortical thickness or area were found between patients and controls in the pre-central (primary motor cortex), the post–central (primary somatosensory cortex) [76], or Brodmann areas (BA1, 2, 3a, 3b, 4a, 4p) (Table 3). Differences in volumes of the caudate nucleus, pallidum, putamen, and thalamus, adjusting for total intracranial volume (TIV), were also not statistically significant between controls and RLS/WED subjects. The results remained negative when age and sex were introduced as nuisance covariates. For subcortical structures, FSpwr showed statistical power over 80% for volume comparison of the thalamus, but lower for caudate (68.5%), pallidum (73.0%), and putamen (76.0%).

4. Discussion

The main finding of this study is that, in a large sample of subjects with RLS/WED evaluated with TMS, SICI was significantly

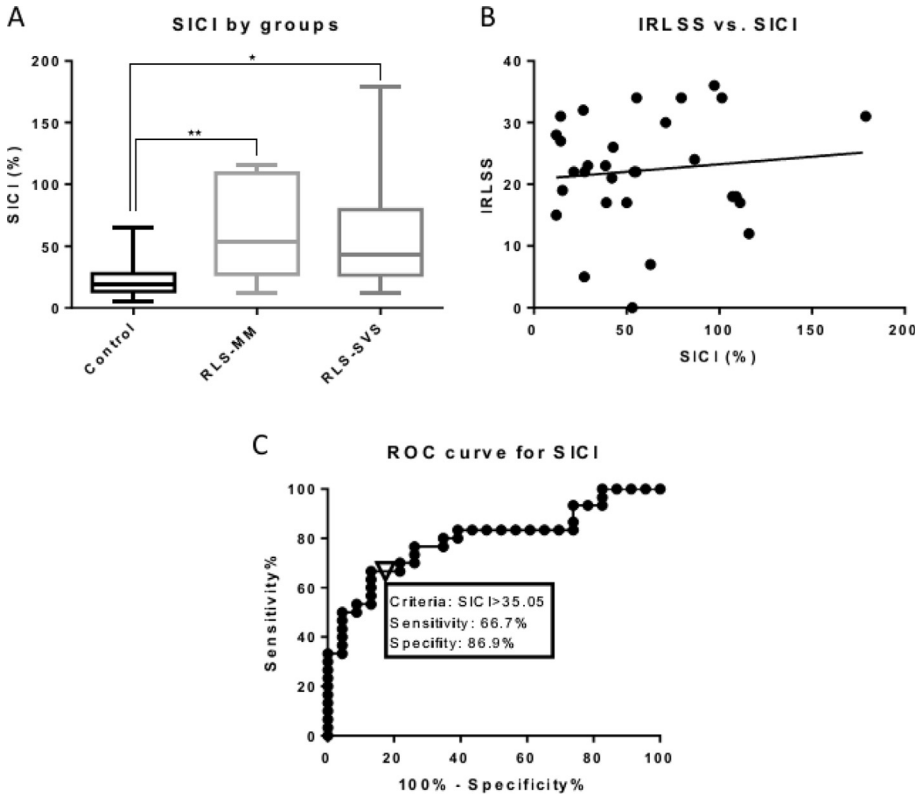

Fig. 1. (A) Short-interval intracortical inhibition (SICI) in patients with restless legs syndrome/Willis-Ekbom disease (RLS/WED) with mild to moderate (RLS/WED_{MM}), and severe to very severe (RLS/WED_{SVS}) forms and controls. (B) Scatterplot/correlation between SICI and International Restless Legs Syndrome Study Group Rating Scale (IRLSS) – $R^2 = 0.019$. (C) SICI ROC curve and cut-off for discriminating RLS/WED subjects and controls. *Post-hoc Tukey (T) = 3.63, $p = 0.002$; **T = 3.66, $p = 0.002$.

Table 3
Comparison of cortical thickness (μm) by hemisphere from surface-based morphometric data using FreeSurfer between controls and idiopathic restless legs syndrome/Willis-Ekbom disease (RLS/WED) subjects included in the morphometric surface-based analysis (Freesurfer v6.0.0).

	Control ($n = 23$)	RLS/WED ($n = 31$)	F	p
Right hemisphere, μm, mean (SD)				
Pre-central area	2470.6 (152.5)	2489.3 (124.0)	0.25	0.622
Post−central area	2044.2 (141.1)	2012.3 (127.6)	0.75	0.389
BA1	2265.5 (230.5)	2265.8 (206.3)	0.00	0.996
BA2	2144.2 (152.9)	2111.8 (148.1)	0.61	0.440
BA3a	1640.9 (135.7)	1634.8 (99.5)	0.04	0.851
BA3b	1610.7 (105.8)	1600.9 (96.0)	0.12	0.728
BA4a	2459.1 (220.1)	2539.3 (162.4)	2.33	0.133
BA4p	2255.2 (215.1)	2305.7 (156.0)	0.98	0.327
Left hemisphere, μm, mean (SD)				
Pre-central area	2522.6 (160.1)	2515.8 (129.1)	0.03	0.864
Post−central area	2080.4 (124.5)	2046.6 (103.9)	1.18	0.282
BA1	2169.4 (210.4)	2174.9 (177.1)	0.01	0.918
BA2	2273.5 (148.4)	2212.7 (135.9)	2.41	0.127
BA3a	1731.9 (155.0)	1697.1 (112.3)	0.90	0.348
BA3b	1837.2 (82.5)	1795.3 (99.1)	2.68	0.108
BA4a	2528.7 (198.6)	2573.5 (150.2)	0.88	0.353
BA4p	2329.4 (249.9)	2335.9 (174.2)	0.01	0.912

BA, Brodmann areas; SD, standard deviation.
[a]*P* Value: one-way analysis of variance.

decreased in RLS/WED subjects with different degrees of severity, compared to controls. These results are in contrast to our hypothesis that only the RLS/WED$_{SVS}$ subgroup would present diminished SICI, indicating that SICI is abnormal even in less symptomatic patients.

We previously reported the lack of differences in rMT, active motor threshold, or cortical silent period between subjects with RLS/WED and controls [57], suggesting that RLS/WED pathogenesis does not involve abnormal neuronal membrane excitability in the motor cortex or in the spinal cord [83,84], or dysfunctional GABA-B receptor activity [85].

Overall, these findings support the hypothesis of a decreased GABA-A activity [24,86] in the motor cortex of individuals with RLS/WED, because SICI is traditionally considered a marker of such activity [62,87−90]; however, this concept has been challenged. Notably, some authors question this theory because of MR spectroscopy studies that did not find a correlation between GABA concentrations and SICI [91]. However, this may be explained by the assessment of phasic GABAergic activity by SICI [91], and by tonic extracellular GABA concentrations by spectroscopy [92,93].

Recently, an elegant and well-designed TMS study [94] by Salas et al., did not show SICI reduction in subjects with RLS/WED. However, at least 13 of 35 patients had history of recent drug treatment (with suspended medication 10 days before the experimental protocol). It is known that dopamine agonists increase SICI in RLS/WED patients, and the duration of this effect has not been determined. Therefore, the inconsistencies between the decrease in SICI in subjects with RLS/WED in the present as well as in previous studies, and the lack of significant difference in SICI between patients and controls in the study by Salas et al. [94], may be attributed to different characteristics of patients in relation to prior exposure to dopamine agonists and possibly other drugs.

Decreased SICI is not specific to RLS/WED and has been reported in other diseases such as Parkinson disease, focal hand dystonia, juvenile myoclonic epilepsy, spinocerebellar ataxias, and motor neuron diseases [61,95,96]. Abnormal inhibition in the motor cortex itself is more likely to explain these findings in juvenile myoclonic epilepsy and motor neuron diseases, whereas cortical changes secondary to abnormal input from subcortical areas are more plausible in the other conditions [61,96]. Although conflicting results have emerged regarding TMS indices of inhibition in major depression, most of the results are consistent with the “cortical disinhibition” hypothesis, in which GABAergic impairment (in terms of reduced SICI) is involved in the pathophysiology of major depression (a recent comprehensive review is provided elsewhere [97]). A decreased SICI has also been found in systemic disorders with peculiar central nervous system (CNS) involvement, such as celiac disease. In particular, de novo celiac patients with subclinical neurological involvement exhibited reduced SICI [98]. It is noteworthy that, SICI did not change after both a short [99] and a long [100] period of gluten-free diet, suggesting that an intracortical synaptic dysfunction, mostly involving the balance between excitatory and inhibitory interneurons within the motor cortex, may persist notwithstanding the diet. In healthy subjects, dopaminergic agonists, such as those used in RLS/WED treatment, increase SICI, whereas dopaminergic antagonists reduce it [101]. It remains to be determined whether the SICI decrease in RLS/WED is secondary to abnormal dopaminergic activity in the striatum, in sensory relays projecting to the primary motor cortex, or to other unknown factors.

Previously, the presence of limb discomfort during TMS testing was reported to be significantly correlated with decreased SICI in subjects with RLS/WED [6]. We found a trend for decreased SICI in the subset of RLS/WED patients who reported leg discomfort either before or after TMS. SICI can be decreased during muscle contraction [102,103], but it is unlikely that incomplete relaxation associated with discomfort explains these results, because in our experimental paradigm TMS stimuli were delivered only when EMG activity at baseline was $\leq$50 μV, for at least 1 s [63]. A plausible explanation for this trend is abnormal sensory processing, considering that SICI decreases after peripheral electrical sensory stimulation in healthy individuals [104]. This hypothesis is further supported by reduced functional MRI connectivity between the thalamus and multiple brain areas, including the pre-central and parahippocampal gyri, in subjects with RLS/WED compared to controls [105]. Moreover subjects with RLS/WED have impaired long term depression (LTD) like mechanisms induced by repetitive transcranial magnetic stimulation, adding support to the involvement of GABA in RLS/WED pathophysiology [106].

We were able to define a “cut-off" magnitude of SICI decrease to discriminate subjects with RLS/WED from controls with moderate sensitivity, high specificity, and acceptable accuracy. However, severity of symptoms was not correlated with SICI, which was decreased across a range of severity. Correlations between SICI and disease severity have been reported in some conditions that involve the motor cortex. In amyotrophic lateral sclerosis (ALS), decreased SICI correlates with disease severity [107]. During progression of spinocerebellar ataxia type 3 (SCA3), in which motor cortex involvement may occur, SICI progressively decreases from the presymptomatic to severe states [108,109]. In both ALS and SCA3, morphometric studies show gray matter thinning or reductions in precentral gyri, supplementary motor areas, thalami, and cerebellum [109−111]. In these diseases, one possible mechanism for reduction in SICI would be a loss of inhibitory cortical interneurons, as neuropathological studies revealed a loss of parvalbumin-positive GABAergic cortical interneurons in patients with motor neuron disorders [112,113].

Our study did not reveal differences in primary motor and somatosensory areas using the FreeSurfer (v.6.0.0) most recent version for SBA, which is reported to have improved segmentation accuracy in relation to its previous releases [114]. We speculate that SICI decrease is unlikely related to cortical motor degeneration, the putative mechanism underlying structural and functional changes in ALS and SCA3. GABA-A dysfunction, indirectly mediated by dopaminergic imbalance in the striatum or by abnormal sensory processing, seems more plausible.

MRI data collection and analysis were performed with state-of-the-art methods. Cortical thickness and area, as well as subcortical volumes measures in FreeSurfer are highly reliable, and the use of 3.0T MR system significantly reduces the within-scanner measurement variability [76,115], which may explain the difference between our findings and those of prior morphometric studies.

Considering the statistical power above 80% in the target regions, the lack of morphometric differences between controls and patients suggests that gray matter structural abnormalities are not relevant to RLS/WED pathogenesis. Most studies that reported decreased or increased gray matter content in RLS/WED in the thalami, hippocampi, primary somatosensory and motor cortices, medial frontal, parietal, and temporal lobes, and cerebellum [28–31] used VBM techniques or FreeSurfer's previous version for data analysis [29]. In addition, most of these studies did not include a measure of TIV as a covariate (recommended because TIV has positive association with volumes [80]) and did not correct their analyses for multiple comparisons. Only one VBM study [32] performed this correction and described slightly increased gray matter density in the left ventral hippocampus ($p = 0.046$) and right middle orbitofrontal gyrus ($p = 0.046$). However, the relation between these regions and mechanisms underlying RLS/WED remains unclear. Indeed, these findings may represent false-positive results, as the data were processed with an older version of the SPM software that lacked the DARTEL algorithm [116] for spatial normalization and optimized the topological false-discovery rate [117]. Furthermore, these results were not reproducible in data analyzed with newer versions of SPM software [34,35,37]. None of the VBM studies that applied corrections for multiple comparisons reported gray matter changes in RLS/WED [28,31,33–37,118].

The only previous SBA study in RLS/WED patients found modest reductions in cortical thickness in central somatosensory regions [29]; however, in this study, the MR acquisition was performed with lower spatial resolution and with an eight-channel head coil. This coil configuration presents lower signal-to-noise ratio than the 32 channel head coil used in our research [119], which significantly improves this ratio in around 40% at the periphery of the brain. Together, these methodological differences could be responsible for the contrasting findings with this paper.

The present study has a few limitations. For TMS, we chose a target muscle in the hand and not in the lower limbs, where RLS/WED symptoms are more frequent. However, decreased SICI has been consistently described in studies that targeted both upper and lower limbs muscles [6,18] or only upper limb muscles [7,20–23,120,121]. Furthermore, TMS coil positioning was performed without the aid of a neuronavigated system [122]. Nevertheless, on a group-level analysis, traditional coil positioning [123] performs similarly to neuronavigated systems [124]. In addition, our SICI protocol included evaluation only at one intensity (80% of rMT) and at one interstimulus interval (2 ms). According to the International Federation of Clinical Neurophysiology (IFCN) guidelines published two years after we had started the study [125], a range of CS intensities could be used to obtain SICI recruitment curves at specific ISIs. The guidelines acknowledge that such a testing protocol is lengthy, that abbreviated protocols are required in many experimental situations, and suggest, for SICI, the choice of ISI 2 ms (as performed in our paradigm) and CS intensity below active MT to avoid contamination with SICF. Therefore, our protocol is in line with the guidelines, was tailored to our goal, and was sensitive enough to detect differences between RLS/WED patients and controls. Furthermore, experiments were performed exclusively during the afternoon (14:00 to 17:00) in all volunteers. Although this adds consistency to our result in terms of circadian variation influence in TMS parameters, we did not assess them during the night, and this could be an important topic for future studies.

Cognitive, clinical, and mood status were assessed through unstructured interviews rather than formally assessed through clinical or psychometric scales. Hence, it is possible that subtle symptoms, which might be relevant in relation to the TMS parameters used here to assess RLS/WED, were not detected. The influence of mood and cognition on the SICI in RLS/WED should, therefore, be explored in more detail in future studies. Moreover, although nocturnal polysomnography is not required in RLS/WED diagnostic criteria except in suspicion of secondary causes or comorbidities, it may be interesting to explore in future studies the correlation between the periodic limb movement index and SICI.

For our SBA approach, MRI power analysis for subcortical structures was marginally underpowered for our study; thus larger samples may yield different results. On the other hand, careful selection of subjects by a neurologist specialized in sleep medicine applying the criteria established by the IRLSSG, the use of a 3.0 T MR system equipped with a 32-channel head coil for high-resolution brain MRI, performing SBA with FreeSurfer version 6.0, and blinded analysis of TMS parameters add methodological rigor and strengthen our findings.

5. Conclusion

In conclusion, SICI was consistently decreased in patients with idiopathic RLS/WED, regardless of disease severity. SICI reduction was present even in subjects with mild symptoms. Cortical thickness and subcortical structure gray matter volumes were normal according to a state-of-art morphometry method. Considering the known complexity of RLS/WED pathophysiology, these results support the concepts of motor cortical disinhibition and preserved cortical structure in this condition.

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Conflict of interest

The authors report no conflict of interest.

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: https://doi.org/10.1016/j.sleep.2019.03.021.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2019.03.021.

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