

Lack of specific gray matter alterations in restless legs syndrome in elderly subjects

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Abstract Magnetic resonance imaging studies using voxel-based morphometry (VBM) have been inconsistent in demonstrating volumetric differences in patients with restless legs syndrome (RLS). Since treatment, age and selection of patients may introduce a methodological bias, we conducted optimized VBM analyses in unmedicated elderly subjects reporting RLS. Two hundred-four volunteers, 65.9 ± 0.6 year-old, free of any significant medical condition and without previous neurological or psychiatric medication, participated at the study. After exclusion of subjects having sleep-related breathing disorders and previous silent infarct, 71 subjects, 54 without RLS (RLS–) and 17 having RLS (RLS+) were analyzed. No structural change in gray matter density was found in RLS+ subjects compared to RLS– subjects. Subjects with RLS+ symptoms showed a small gray matter volume in the left occipital region without, however, statistical significance.

VBM analysis did not show any significant change in subcortical and cortical gray matter in unmedicated elderly subjects with RLS symptoms. These results confirm the lack of specificity of thalamic and subcortical changes in restless legs syndrome.

Keywords Restless legs syndrome · Elderly · Neuroimaging · Voxel-based morphometry · Pathophysiology

Introduction

Restless legs syndrome (RLS) is a sleep disorder characterized by uncomfortable leg sensations, usually prior to sleep onset or during the night, that cause an irresistible urge to move the legs [1]. The RLS's prevalence is estimated at 5–20% [14] with increased rate in elderly [15]. Neuroimaging studies using different techniques [13], i.e. functional magnetic resonance imaging (fMRI), single photon emission computed tomography and positron emission tomography, have attempt to localize cerebral generators of leg discomfort and periodic limb movements in RLS, and the results are somewhat conflicting [19]. Using high-resolution fMRI, Bucher and co-workers [4] showed a bilateral activation of the cerebellum and contralateral activation of the thalamus when patients with RLS experienced leg discomfort, and an additional activation of red nuclei when a periodic limb movement occurs. These findings suggest that subcortical cerebral generators are involved in the pathogenesis of RLS, thalamus and cerebellum primarily involved in the sensory processing, and red nuclei and brainstem areas in the genesis of motor pattern. In line with this investigation, a recent voxel-based morphometry MRI study reported a bilateral gray matter

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increase in the pulvinar in 51 treated patients [5], confirming the thalamic dysfunction in RLS patients. Moreover, Astrakas and co-workers [2], using magnetic resonance relaxometry, confirmed during a motor paradigm the activation of the thalamus, putamen and middle frontal gyrus in RLS patients. More recently, Lee et al. [9] showed that in patients having stroke-related RLS, lesions of the thalamus and pons and/or the basal ganglia-brainstem axis were present, indicating a subcortical involvement in secondary RLS. Therefore, a neuroanatomical model for RLS has been proposed suggesting a primary subcortical involvement of brainstem, thalamus and cerebellum and a dysfunction of the dopamine system [19]. However, conflicting results have recently been reported, one study [8] showing no alteration in thalamic areas in 14 untreated idiopathic RLS patients and others [17, 18] reporting significant gray matter decreases in the primary somatosensory cortex and white matter tract changes in somatosensory cortices, thalamus and cingulum. The last results indicate an altered sensorimotor network in RLS patients more than a specific area of lesion.

The conflicting results on morphological brain changes in RLS patients might be attributed to differences in either the population studied or the methodology used. Differences in study populations potentially affecting results include the age and the treatment status, the changes in subcortical and thalamic structures being more a consequence of dopaminergic therapy [5] or a brain gray matter volume change related to aging and associated medical disorders [14, 15]. Differences in methodologies can include differences in MRI acquisition protocols, definition of the region of interest and the selection of region of interest. Region of interest (ROI) validation requires demonstration that the region of change is localized within the region of interest and does not involve adjacent or contiguous brain areas.

An approach that avoids the problems of methodology and populations is a survey study of the incidence of RLS in an unselected population using the voxel levels. The objective of the present study was to use optimized voxel-based morphometry (VBM) analysis to compare regional gray matter volumes in an elderly sample with and without RLS matched for age. This unbiased approach was chosen to identify specific volumetric alteration of subcortical areas and thalamus in elderly subjects with RLS.

Methods

Population

Our study is a part of the synapse study conducted in elderly volunteers, all residents from the Saint-Etienne urban district (France) included at their 65th anniversary to assess the

contributing role of sleep-related breathing disorders (SRBD) and RLS on quality of life, cognitive function and brain morphology. For the actual study, exclusion criteria included (1) significant medical condition including myocardial infarction, heart failure, stroke, type 1 diabetes, previous diagnosed neurological or psychiatric diseases such as Parkinson's or other neurodegenerative disease, evolutive cancer, hemodialysis, (2) subjects dependent or living in institutions such as nursing homes, (3) presence of sleep related breathing disorders as assessed by nocturnal polygraphy and defined as an apnea plus hypopnea index (AHI) >15 [12], (4) presence of psychotropic and dopaminergic medication, and (5) presence of silent infarcts or other structural abnormalities on the 3D MRI recording. Of the original sample of 204 subjects, 71 subjects, 50 females and 21 males, aged 65 years meet the study criteria. The study was approved by the local ethics committee (CCPPRB Rhône-Alpes Loire) and all subjects gave written consent to study participation.

Restless legs syndrome score

As an indication of RLS, we used the minimal diagnostic criteria proposed by the international RLS study group [1] i.e. complaint of unpleasant sensation in the legs, urge to move, relief by movements, worsening in the evening or at night. We used the additional criteria of frequency of symptoms occurring once per week during the last 6 months in order to facilitate exclusion of subjects having symptoms mimicking RLS [3] more frequent in older population. Subjects answering affirmatively to all five basic symptoms were assigned as having RLS (RLS+) and received detailed and individual instructions regarding how to answer the International Restless Legs Syndrome Study Group (IRLSSS) severity score questionnaire [16].

Sleep study

Nocturnal unattended sleep study was done in all subjects using a polygraphic system (HypnoPTT; Tyco Healthcare, Puritan Bennett) which included sound measurement, electrocardiography, pulse transit time, R–R timing, nasal pressure, respiratory effort, body position and oxygen saturation (SaO₂). The apnea + hypopnea index (AHI) was established as the ratio of the number of apneas and hypopneas per hour of recording. As indices of nocturnal hypoxemia we considered the mean SaO₂, the minimal value recorded during sleep and the oxygen desaturation index (ODI). Pulse transit time was continuously monitored and an autonomic arousal index (AAI) was calculated. An AHI >15 was considered as a diagnostic of obstructive sleep apnea syndrome according to criteria previously applied in the elderly [12].

Magnetic resonance imaging (MRI)

Brain scans were acquired on a Siemens 1.0 Tesla scanner. For each subject, a 3D T1-weighted MRI (MPRage) was acquired with the following parameters: TR = 1,900, TE = 3.95, FOV = 256 × 256, 88 slices per volume with a voxel size of 2 × 2 × 2 mm. T2-weighted (24 slices of 5.5 mm, TR = 6620, TE = 123, FOV = 173 × 230, pixel size : 1.5 × 0.9 × 5.5 mm) and FLAIR (24 slices of 5.5 mm, TR = 9,000, TE = 102, FOV = 230 × 173, pixel size : 0.9 × 0.9 × 5.5 mm) scans were also recorded in the same MRI session. All MRIs were analyzed by an experienced radiologist blinded to the sleep study results.

Voxel-based morphometry

All brain scans were processed by using Statistical Parametric Mapping (SPM2; Wellcome Department of Cognitive Neurology, London, UK) and the VBM method using the optimized protocol [6] for VBM brain analysis. A study-specific template was first created using 61 visually normal MRIs scans. To avoid misregistrations, all scans were then segmented, normalized to this template and segmented again. To study volume differences, all resulting scans were modulated and smoothed by a 12-mm FWHM kernel. Finally, a complementary analysis focused on ROI, i.e. thalamic and frontal areas, was done according to WFU_PickAtlas v2.4 [11].

Statistical analysis

Subjects with and without RLS were compared using ‘multi-group: covariates only’ model in SPM2. Gender and total gray matter volume were introduced as covariates. Thresholds for significant level were set at $P < 0.01$ with a False Discovery Rate correction.

Two-tailed unpaired Student’s *t* tests were used to compare population clinical and demographic data between RLS– and RLS+ subjects. Classical statistical analyses were done with Statistica 6.1 (Statsoft) and results are presented as mean ± SD. Statistical significance was achieved for *P* values smaller than 0.05.

Results

Of the original study group of 71 subjects, 54 (34 females and 20 males) were considered as RLS– and 17 subjects (16 females and 1 male) considered as RLS+. The mean severity IRLSSS score was 15.7 ± 4.8, no subject having very severe form of the disease.

Table 1 shows the clinical, anthropometric and polygraphic data for the group with (RLS+) and without

(RLS–) RLS. Subjects with and without RLS did not differ in terms of age, BMI, neck circumference, hypertension status, glycemia, depression, anxiety, AHI, ODI, and AAI.

Results of VBM analyses are summarized in Table 2 and Figs. 1 and 2. No significant difference in volume or density was found in thalamic area, hippocampus and frontal area (Table 2). The lack of differences still remain when gender was introduced as a cofactor. There was a tendency for RLS– subjects to have a smaller gray matter volume in the left occipital region (Fig. 1) and for RLS+ subjects to have a smaller gray matter volume in the right temporal area (Fig. 2), but the differences did not reach statistical significance. ROI analysis confirmed the lack of differences between groups for specific thalamic and frontal areas.

Discussion

Previous structural MRI studies using VBM analyses in RLS subjects have found thalamic gray matter changes in RLS patients [5], alterations not confirmed by other studies [8, 17]. The conflicting results on the MRI structural changes might be attributable either to methodological differences, such as image resolution and optimized VBM analysis [6, 13] either to study population, coexistence of other sleep disorders [10] and age [19], factors inducing brain anatomical or functional alterations. Examining a sample of healthy elderly matched for age and without dopaminergic treatment we found the lack of structural thalamic and subcortical gray matter differences in subjects with RLS. In line with previous observations [8], our findings suggest that the previously reported thalamic involvement may be a consequence of chronic increase in afferent input or a consequence of dopaminergic medication [7, 8] inducing a disruption in pulvinar circuitry.

Table 1 Clinical and polygraphic parameters for the total sample and for subjects with (RLS+) and without (RLS–) restless legs syndrome (means ± SD)

	RLS–	RLS+	<i>P</i>	Population
Number of subjects	54	17		71
Gender (M/F)	20/34	1/16	0.014	21/50
Age (years)	66.0 ± 0.5	65.9 ± 0.8	0.50	66.0 ± 0.6
BMI (kg m ⁻²)	24.5 ± 2.6	24.7 ± 3.6	0.80	24.5 ± 2.8
AAI	42.6 ± 25.8	39.1 ± 16.5	0.63	38.9 ± 25.6
AHI	8.3 ± 4.3	8.3 ± 4.2	0.99	8.3 ± 4.2
ODI	3.9 ± 3.2	4.8 ± 3.7	0.32	4.1 ± 3.3
ESS	5.2 ± 3.6	6.1 ± 4.2	0.43	5.4 ± 3.7

AAI autonomic arousal index, AHI apnea plus hypopnea index, BMI body mass index, ESS Epworth sleepiness scale, ODI oxyhemoglobin desaturation index

Table 2 Regions of gray matter differences between RLS– and RLS+ subjects

X	Y	Z	P (cluster level)	Location
Regions where RLS– have smaller gray matter volume than RLS+				
–24	–98	–6	0.931	Left inferior occipital
–2	–92	10	0.963	Left calcarine
–10	–98	–10	0.896	Left calcarine
Regions where RLS+ have smaller gray matter volume than RLS–				
56	–12	0	0.303	Right superior temporal

Moreover no difference in sensorimotor cortex [17, 18] and in hippocampus and middle orbitofrontal areas [8] was found between groups. The slight differences in temporal and occipital areas between our RLS+ and RLS– subjects do not appear to be specific to RLS, the differences being slight and not reaching strong statistical significance. Therefore, we can conclude that the use of optimized VBM, the analysis of ROI and the selective population inclusion criteria allow us to confirm the lack of specific thalamic involvement in unmedicated elderly RLS subjects. The lack of significant alteration in other brain areas in our subjects opens question on accuracy of previous reports, the cortical, subcortical and thalamic changes probably more a casual finding related to other factors outside RLS than a real and specific brain alteration related to the sensorimotor pathology.

Some limitation of our study should be considered in the interpretation of results. First, our population derived by an elderly unmedicated population in that diagnosis of RLS was made according to questionnaires. Despite the lack of polysomnographic or actigraphic assessment, we are confident that the used questionnaires allow diagnosis of RLS, clinical finding being the cornerstone of RLS diagnosis [1]. Second, our subjects have an IRLSSS score in the mild to

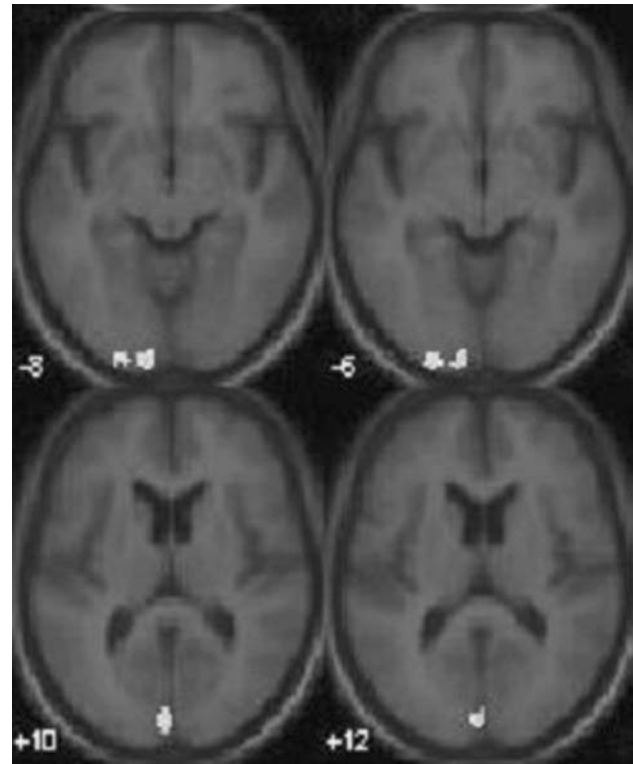


Fig. 2 Regions where RLS– subjects have smaller gray matter volume than RLS+. Results are displayed with a $P < 0.01$ uncorrected threshold. Gray matter differences disappear with a confident threshold

moderate range in contrast to previous studies [5, 8, 17] in that severe or very severe cases were examined. Although speculative and not supported by previous studies, one possible explanation is that in elderly RLS severity is lower compared to middle aged subjects as a possible adaptation of older patients to chronicity of symptomatology. This would also explain why our healthy subjects were not treated and no diagnosed at the time of inclusion. Finally,

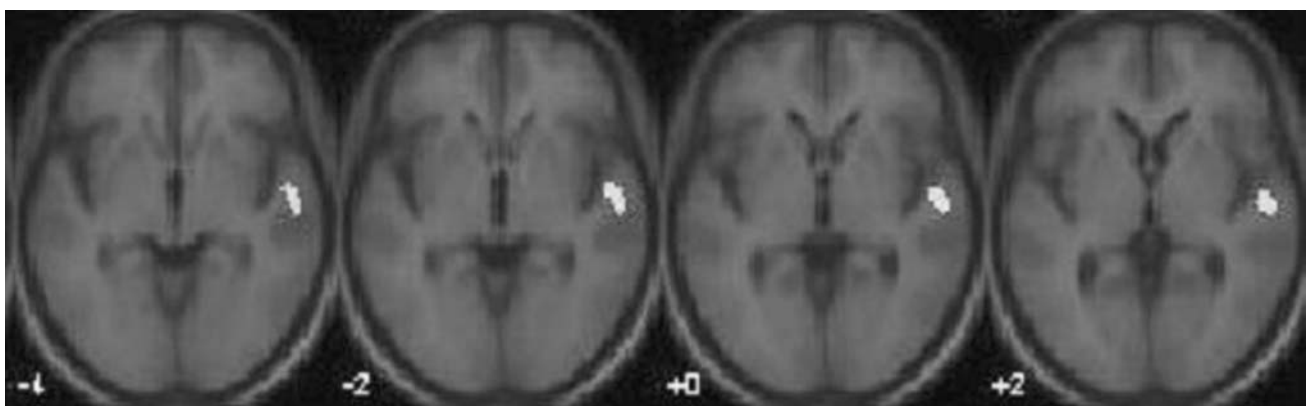


Fig. 1 Regions where RLS+ subjects have smaller gray matter volume than RLS–. Results are displayed with a $P < 0.01$ uncorrected threshold. Gray matter differences disappear with a confident threshold

we knew that age and gender may affect symptomatology [1] and fMRI data [17], factors that might contribute to difference in published results. We choose to examine a homogenous group of subjects matched for age without any dopaminergic and psychotropic medication, which rules out the effect of age and medication in our results. Even though in our sample there was a high incidence of women, probably related to exclusion of subjects having sleep-related breathing disorders, i.e. men, the introduction of gender as covariate did not affect our results.

In conclusion, examination of unmedicated RLS subjects, without other neurological, psychiatric and sleep disorders, does not support the hypothesis of thalamic involvement in restless legs syndrome. Future research needs to be done in order to analyze if neural networks dysfunction more than specific brain generators are implicated in the sensorimotor symptoms of RLS.

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