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Late-onset restless legs syndrome evaluated at night-hours with resting-state fMRI of the brain and voxel-based morphometry

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ABSTRACT

Purpose: The aim of the study was to investigate the resting state networks and brain volumetric changes in patients with late onset restless legs syndrome (lRLS).
Material and Methods: Nine untreated lRLS patients (mean age 65.2 +/- 7.7; mean disease duration 4.2 +/- 2.6 years) and 9 age and sex matched controls were evaluated at night hours with resting state fMRI (rs-fMRI) and a 3D T1-weighted sequence. The evaluation of rs-fMRI data was performed using the independent component analysis model. Volumetric changes were evaluated with voxel based morphometry (VBM).
Results: The fMRI analysis revealed eight resting state networks. Patients compared to controls showed significant greater activation of the supplementary motor area and the premotor cortex in the right hemisphere. VBM analysis did not reveal any significant volume change in brain white and grey matter.
Conclusions: Untreated patients with lRLS do not have any regional brain volume alterations. Activation in the presymtomatic period of the supplementary motor cortex and the premotor cortex may represent anticipation of the forthcoming sensorimotor symptoms.

KEY WORDS

functional magnetic resonance imaging; restless legs syndrome; premotor area; supplementary motor area

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1. Introduction

The restless legs syndrome (RLS) is a common sensorimotor disorder characterised by sensory uneasiness that evokes an urge to move the limbs while attempting rest, usually at night [1]. RLS may be idiopathic or secondary to medication or disease, including Parkinson's disease, peripheral neuropathy and certain autoimmune disorders. Idiopathic RLS encompasses an early-onset (*e*RLS) and a late-onset (*l*RLS) phenotype [2]. The pathophysiology remains unclear but MRI studies have demonstrated T2 relaxometry metrics compatible with a low iron content in the substantia nigra in patients with *e*RLS and *l*RLS [3-7]. Abnormal brain iron content has often been associated with neurodegeneration and atrophy [8]. Voxel-based morphometry (VBM) studies in RLS patients have yielded inconstant alteration of brain volume [1, 9-15]. This lack of consistency between the results of different studies is probably attributed to inhomogeneous populations comprising *e*RLS and *l*RLS, treated and non-treated patients as well as to the different methods of data analysis. There is only one study assessing a homogeneous sample of patients of *e*RLS patients with VBM analysis [1]. There are no VBM studies focusing on patients with *l*RLS.

The blood oxygenated level dependent (BOLD) signal, measured by fMRI, depicts the brain regions that are active while a patient performs a motor or sensory paradigm [6] or during rest [16]. Functional MRI (fMRI) studies have been conducted in RLS patients with either undefined [17] or defined disease onset [1, 6]. *l*RLS patients have been evaluated with fMRI during imitation of periodic limb movement (PLM) and *e*RLS patients, evaluated with fMRI at night during episodes of exacerbation of symptoms, revealed activation of the striatofrontolimbic system [1]. Resting state fMRI (rs-fMRI) identifies multiple spatially distinct areas of the brain that demonstrate synchronous BOLD fluctuations at rest. These functionally connected areas during the resting state are defined as resting state networks (RSNs) [16, 18]. Because the symptoms of RLS present at rest, evaluation of patients with rs-fMRI would be useful to search for abnormalities in resting state networks that might provide insight in the pathophysiology of this syndrome. There are very few rs-fMRI studies in patients with idiopathic RLS and in none of them distinction was made between *l*RLS and eRLS [19, 20]. One study was performed in drug naïve patients at early time hours and the second in patients treated with levodopa at night time hours [19, 20]. Our hypothesis was that evaluating patients at night hours before the appearance of symptoms would enhance abnormalities in the RSNs related with the appearance of symptoms.

The aim of the study was to evaluate with rs-fMRI untreated *l*RLS patients during wakeful rest at night hours and to assess for brain volume changes at a voxel basis.

2. Material and Methods

Over a two-year period (June 2009-June 2011) we assessed 32 consecutive patients suffering of RLS, with either late or early onset, employing an fMRI protocol with the aim to explore the networks that are active during periodic limb movements. Yet, retrospectively, those patients that did not move during the fMRI were considered to consist of a homogeneous group that merited to be studied for the recording of resting state networks. Therefore, 9 right-handed patients with idiopathic *l*RLS (5 women, 4 men; age range 54-77 years; mean age 65.2 +/- 7.7 years; mean disease duration 4.2 +/- 2.6 years) were analysed and compared to 9 sex- and age-matched right-handed controls. All the patients had late disease onset with symptoms starting after 45 years of age. None of the patients had received dopaminergic drugs. Lateralisation of the symptoms was not reported. Every subject underwent a detail history survey, a physical examination and laboratory exams and willingly agreed to participate in the MR study signing a written consent form. The patients matched the revised essential criteria for the diagnosis of idiopathic RLS defined by the International RLS Study Group (IRLSSG) and supportive diagnostic criteria were also covered by the study. The IRLS is a set of 10 questions based on: a) features of RLS, including intensity (5 items) b) frequency (1 item) of the symptoms and c) consequences of RLS (i.e. sleep quality, daytime tiredness, mood and quality of life) [21]. No polysomnographic study was performed to detect periodic limb movements (PLMs) in sleep, but their presence was based on the history survey. All patients presented PLMs and reported night-aggravated symptoms. Serum ferritin levels were measured on the day of the MRI examination and were within normal values for all participants. Imaging was performed at night hours when patients were typically symptomatic, starting from 9:00 pm. The patients were advised to keep their eyes closed during the fMRI examination so as to minimise potential

visual stimuli and were not given any instructions. Patients were under continuous surveillance by a technologist and potential leg movement was recorded during the fMRI sequence.

2.1 Imaging protocol

MRI data were acquired using a 1.5-Tesla scanner (INTERA, Philips Medical Systems, Best, The Netherlands). The imaging protocol in both patients and control subjects consisted of: 1) a T1-weighted high-resolution (0.86 x 0.86 x 1 mm) three dimensional spoiled gradient echo sequence (TR/TE, 25 ms/4.6 ms), which was used for structural imaging; and 2) a single shot multislice gradient echo planar imaging (EPI), which was used for BOLD functional images (TR/TE, 3,000 ms/50 ms; flip angle, 40°, matrix, 64 x 64; slice thickness, 5 mm; gap, 0 mm). Each fMRI session consisted of 160 scans and lasted 480 seconds. At the beginning of each session, four dummy scans were acquired to allow equilibration of magnetisation. The head of the subject was restrained using cushions to minimise motion artefacts. The patients were instructed to remain lying during the examination and to move if they experienced the usual uncomfortable sensations. Potential PLMs were recorded in real time by a technician with direct visual contact with the patient using Lumina LP-400 patient response system and Superlab 4.0 stimulus presentation software (Cedrus Corporation, San Pedro, CA). The fMRI paradigm was customised to assess brain activity of the patients with RLS during the time they usually started having uncomfortable leg sensations and was applied similarly to the normal control subjects at night, starting from 9:00 pm.

2.2 Image analysis and statistical analysis

VBM method using the unified segmentation approach of the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) was implemented using the T1-weighted images [22]. A scanner-specific template of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) compartments was constructed on measurements of 24 healthy subjects (12 women, 12 men; mean age: 58.9 +/-10.6 years). VBM involved simultaneous normalisation of all images according to the scanner-specific template, correction for intensity inhomogeneity and segmentation of GM, WM and CSF compartments [22]. Morphological differences between patients with *l*RLS and control subjects were estimated using independent samples t-test at the voxel level with a false discovery rate (FDR) adjusted p value of 0.05. The comparison between patients and control subjects was made for two different contrasts corresponding to increase (*l*RLS>controls) or decrease (*l*RLS<controls) of brain volume in the GM and WM compartments.

FMRI data analysis was performed using independent component analysis (ICA), a well-documented exploratory method for analysing complex data sets from fMRI experimental studies. Unlike regression-based methods, such as the general linear model, ICA does not require a predefined model and thus represents a data-driven analysis approach [23]. ICA manipulates the 4D data as the sum of a set of spatiotemporal components, each of which consists of a spatial map modulated in time by a relative time-course. Different components are separated under the assumption that the spatial maps are statistically independent of each other and have different time-courses. Each component ideally represents a different artefact or activation pattern. ICA has been applied in numerous fMRI studies [23-26].

Image datasets preprocessing was performed using SPM8, including realignment for motion correction, slice-timing, spatial normalisation and spatial smoothing using a 8 x 8 x 8 mm^3 full width Gaussian kernel at half-maximum. Structural and functional data of each participant were transformed to standard Talairach stereotaxic space. ICA was performed using the Group ICA of the fMRI Toolbox (GIFT), version 1.3 h (http://icatb.sourceforge.net).

Group ICA analysis requires that all tasks are analysed at once. For each subject the fMRI data were split in two separate datasets, one for the control group and the other for the *l*RLS group. All the preprocessed data from both the control and the *l*RLS group were analysed together in a single group ICA framework [23]. To decrease computational complexity, GIFT implements a two-step method for data compression using principal component analysis (PCA) [27]. In the first step, data from each group were reduced from 100 (the number of time points within the experiment) to 24 dimensions. The reduced data were then concatenated temporally and reduced again to the final 20 components. The average variance retained at each of these steps was 99.96% and 99.94% respectively. This number of components was found optimal using a modified minimum description length (MDL) algorithm [23]. A group spatial ICA is performed on this final

set using the infomax algorithm [28]. The resulting mixing matrix was subsequently used to back-reconstruct the spatial maps and the time courses for each individual subject and each component. Thus, for our data set of 18 subjects with one experimental design, GIFT produced 720 (18 subjects x 20 components x 2 experimental designs=720) spatial maps, with their corresponding ICA time courses.

The ICA components were then normalised using z scores [29]. The former normalisation step is important to ensure that a high z score within spatial maps represents a stronger representation of the ICA time course and vice versa. Temporal ICA components were low frequency spontaneous oscillations, very similar to those of resting state networks [30].

3. Results

T1 and T2-weighted images showed no structural abnormality of the brain in any study subject. VBM analysis revealed no significant differences in brain volume between the patients with *l*RLS and control subjects.

All patients and controls were awake during the fMRI examination and no periodic limb movement was recorded. ICA analysis revealed the presence of 20 spatial components. Each of the 20 components was visually inspected for the presence of artefacts. Nine of the components corresponded to subjects' motion or were spatially localised to cerebrospinal fluid and blood vessels. Out of the 11 resulting independent components, a set of 8 spatial patterns known to be associated with resting state networks were recognised [31, 32].

Comparison between the SPM maps patients with *l*RLS and controls revealed greater activation of subregions of the component 19 (**Fig. 1**), (coordinates 47 7 44, coordinates 54 11 39) on the right hemisphere in the patients (statistical threshold of $p<0.001$). These areas correspond to the supplementary motor area (SMA) and the premotor cortex (PM) (**Fig. 2**).

4. Discussion

In this study, patients with *l*RLS and control subjects were evaluated at night hours with fMRI and VBM. The major findings in RLS patients were increased activation of the SMA and PM. VBM did not reveal any volume change.

The cardinal finding of RLS is uncomfortable sensations in the limbs described as "creeping, crawling tingling, pulling, or painful" [33]. These uncomfortable sensations are always accompanied by an urge to move the limbs and particularly the legs, leading to a temporary often partial relief of the symptoms [33]. About 80% of patients with RLS also present periodic limb movements (PLMs), which are involuntary movements appearing when the patient is awake or at asleep [34]. Disturbance in brain iron homeostasis and dysfunction in the dopaminergic system has been reported in patients with RLS [34]. Some relief of RLS symptoms may be obtained with dopaminergic medications and histological and MRI studies have been reported in patients with RLS [6, 34, 35]. Low iron content in the SNc may affect the synthesis of dopamine which plays a key role in the cortico-basal-ganglia circuitry that controls motion [36]. The organisation of the cortico-basal-ganglia circuitry comprises an indirect inhibitory and a direct excitatory pathway which, under the influence of dopamine, controls in balance the activity of the globus pallidus internal (GPi) and the substatia nigra pars reticulata (SNr) [37] (**Diagram 1**). At rest the GPi and the SNr send spontaneous inhibitory GABAergic signals to the ventrolateral nucleus (VL) of thalamus and thus inhibit the excitatory signals of VL to the motor cortex [37]. The end result is lack of motion at rest. In RLS low iron in the

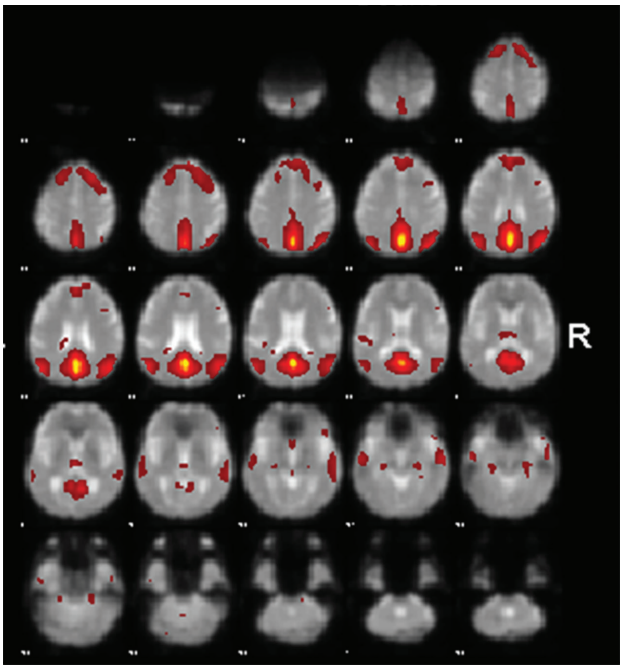

Fig. 1. *The map of component 19 in colour overlaid on a fMRI brain scan.*

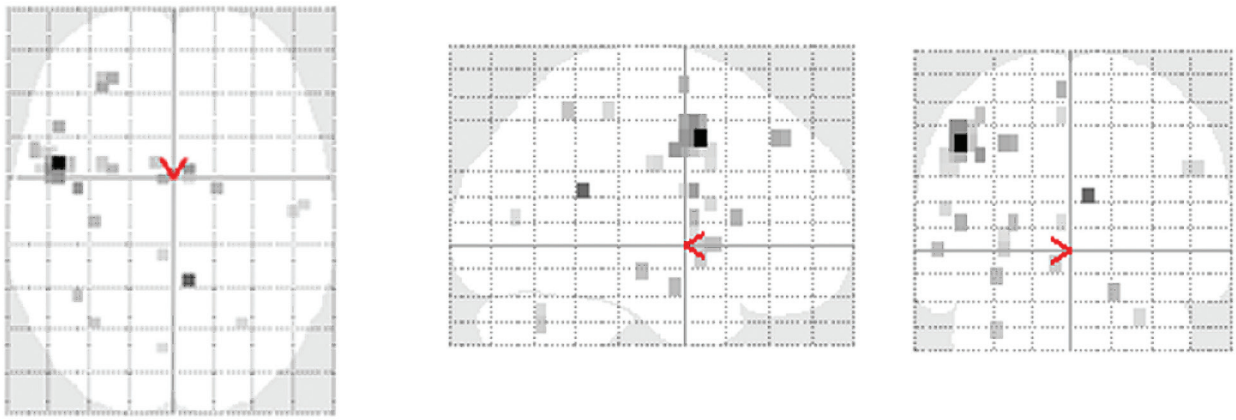

Fig. 2. *Glass-brain representation of the T-statistics map thresholded at P<0.001, depicting the results of the comparison of the component 19 between RLS and controls (RLS>controls).*

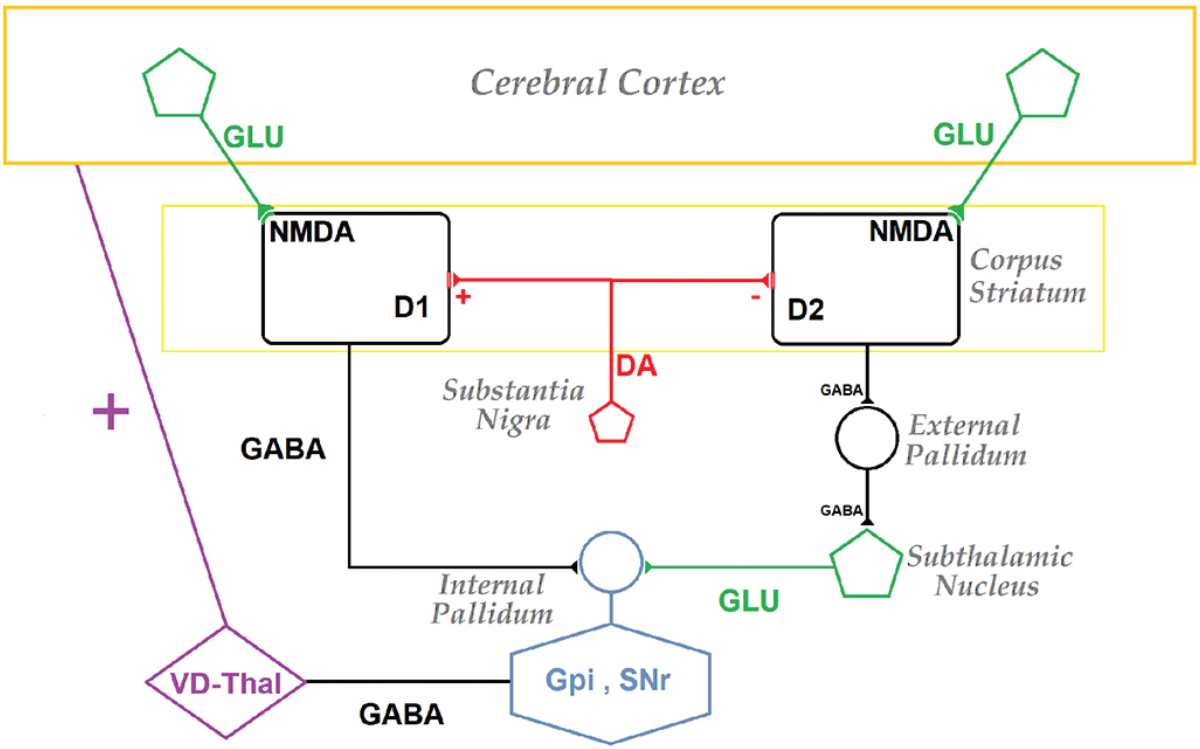

Diagram 1. *A diagrammatic representation of the neuronal circuit of motion that comprises of the direct and the indirect pathway.*

SNc might influence the balance between the indirect and direct pathway and decrease at rest the inhibitory control of GPi and SNr to the VL. This may result in a lower threshold for initiation of movement to the extent that involuntary movements may arise. Abnormal involuntary movements may also arise when increased quantity of dopamine is available to the striatum. Patients with Parkinson's disease treated with levodopa may develop dyskinesias, which are abnormal involuntary movements induced by the excess of dopamine [37]. Increased activation of the SMA in resting state has been reported in patients with Parkinson's disease who would develop levodopa induced dyskinesias [38]. In the present study all patients had involuntary movements and

presented an activation of the SMA before the development of sensory-motor symptomatology. The SMA and PM have been implicated in the preparation of motion [39-42]. Activation of the SMA has also been reported in presence of a conditioned stimulus inducing motion and in motor imagination [39-41]. Patients with RLS in the latent presymptomatic period may activate the SMA and PM by awaiting and imagining motor reactions in response to the expected sensory discomfort. The results of the present study are different compared to those of the two previous studies [19, 20]. This is probably related with differences in the selected populations (*l*RLS *vs* mixed *l*RLS and eRLS, drug naive *vs* treated patients) [19, 20] and in methods of rs-fMRI data processing (independent component analysis versus seed based approach) [19, 20]. Seed based correlation analysis applied in previous studies and ICA in this study are the two major methodologies for exploring functional connectivity of the brain. Their major difference is that the seed based methods provide a single metric of functional connectivity, whereas ICA can additionally decompose the total connectivity into connectivity within each network and connectivity between pairs of networks, based on the more realistic assumption that the same brain areas participate in many different networks [43].

In the current optimised VBM study no significant changes in grey or white matter volume was found between patients and controls. Previous neuroradiological studies of brain volume alterations using volumetric and diffusion tensor methods have been controversial [10, 11, 13]. Inconsistency was estimated to be a nuisance effect of either a heterogeneous sample or of the use of different techniques. Our results are in line with optimised volumetric studies conducted in unmedicated patients with RLS [1, 12, 15]. The current work reinforces the hypothesis that other factors, such as the medication treatment, may have modulated brain volume alterations demonstrated in previous studies.

To conclude, unmedicated patients with *l*RLS do not have regional brain volume alterations and the latent non-symptomatic period may represent the awaiting of the forthcoming sensorimotor symptoms. R

Conflict of interest

The authors declared no conflicts of interest.

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