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Abnormal medial thalamic metabolism in patients with idiopathic restless legs syndrome

Giovanni Rizzo,[1,2,3] Caterina Tonon,[1] Claudia Testa,[1] David Manners,[1,4] Roberto Vetrugno,[3] Fabio Pizza,[2,3] Sara Marconi,[2,3] Emil Malucelli,[1] Federica Provini,[2,3] Giuseppe Plazzi,[2,3] Pasquale Montagna[3],* and Raffaele Lodi[1]

1 Functional MR Unit, Department of Internal Medicine, Aging and Nephrology, University of Bologna, Bologna, Italy
2 IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy
3 Department of Neurological Sciences, University of Bologna, Bologna, Italy
4 Department of Histology, Embryology and Applied Biology, University of Bologna, Bologna, Italy

*Deceased

Correspondence to: Raffaele Lodi, MD, PhD,
Department of Internal Medicine,
Aging and Nephrology,
University of Bologna,
Via Massarenti 9,
40138 Bologna, Italy
E-mail: raffaele.lodi@unibo.it

Pathophysiology of restless legs syndrome is poorly understood. A role of the thalamus, specifically of its medial portion which is a part of the limbic system, was suggested by functional magnetic resonance imaging and positron emission tomography studies. The aim of this study was to evaluate medial thalamus metabolism and structural integrity in patients with idiopathic restless legs syndrome using a multimodal magnetic resonance approach, including proton magnetic resonance spectroscopy, diffusion tensor imaging, voxel-based morphometry and volumetric and shape analysis. Twenty-three patients and 19 healthy controls were studied in a 1.5 T system. Single voxel proton magnetic resonance spectra were acquired in the medial region of the thalamus. In diffusion tensor examination, mean diffusivity and fractional anisotropy were determined at the level of medial thalamus using regions of interest delineated to outline the same parenchyma studied by spectroscopy. Voxel-based morphometry was performed focusing the analysis on the thalamus. Thalamic volumes were obtained using FMRIB's Integrated Registration and Segmentation Tool software, and shape analysis was performed using the FMRIB Software Library tools. Proton magnetic resonance spectroscopy study disclosed a significantly reduced *N*-acetylaspartate:creatine ratio and *N*-acetylaspartate concentrations in the medial thalamus of patients with restless legs syndrome compared with healthy controls ($P < 0.01$ for both variable). Lower *N*-acetylaspartate concentrations were significantly associated with a family history of restless legs syndrome ($\beta = -0.49$; $P = 0.018$). On the contrary, diffusion tensor imaging, voxel-based morphometry and volumetric and shape analysis of the thalami did not show differences between the two groups. Proton magnetic resonance spectroscopic findings in patients with restless legs syndrome indicate an involvement of medial thalamic nuclei of a functional nature; however, the other structural techniques of the same region did not show any changes. These findings support the hypothesis that dysfunction of the limbic system plays a role in the pathophysiology of idiopathic restless legs syndrome.

Introduction

Restless legs syndrome is a sensorimotor disorder characterized by an irresistible urge to move the legs, associated with unpleasant paraesthesias in the legs and sometimes in the arms. These sensations occur at rest, in particular in the evening or at night, and are relieved by movement. Symptoms are typically attenuated by dopaminergic drugs (Trenkwalder *et al.*, 2005). The pathophysiology of restless legs syndrome is poorly understood. Several observations point towards an involvement of central nervous structures and networks, but the areas involved are somewhat uncertain. In the first functional MRI study of restless legs syndrome, Bucher *et al.* (1997) reported an activation of the thalamus (leg discomfort), cerebellum (leg discomfort and periodic limb movements), red nuclei and brainstem (periodic limb movements). A more recent functional MRI study, using only a motor paradigm, found activation in the thalamus, putamen, middle frontal gyrus and anterior cingulated gyrus in patients with restless legs syndrome (Astrakas *et al.*, 2008). Among these regions, the anterior cingulate gyrus and thalamus (specifically of its medial portion), which are parts of the limbic system and have different functions including a central role in pain processing, as a part of the medial nociceptive system (Price, 2000), were identified. Nociception involvement in restless legs syndrome is suggested by the possible painful sensations reported by patients with restless legs syndrome (Hornyak *et al.*, 2011; Karroum *et al.*, 2012), the response to opioids (Trenkwalder *et al.*, 2008), and by the link between dopamine and pain control (Millan, 2002). Furthermore, PET studies documented higher D2 receptor radioligand [11C]FLB 457-binding potential (Cervenka *et al.*, 2006) and a negative correlation between the opioid receptor radioligand [11C]diprenorphine-binding and clinical severity (von Spiczak *et al.*, 2005) at the level of limbic areas serving the medial pain system in patients with restless legs syndrome.

The aim of this study was to evaluate the metabolism and structural features of medial thalamic regions in patients with idiopathic restless legs syndrome using a multimodal magnetic resonance approach, including proton magnetic resonance spectroscopy (1H-MRS), diffusion tensor imaging (DTI), voxel-based morphometry (VBM), volumetric and shape analysis of the thalami.

Materials and methods

Subjects

We performed a cross-sectional study. Twenty-six patients with idiopathic restless legs syndrome were recruited from May 2010 to March 2011 by the Sleep Medicine Centre of the Department of Neurological Science of Bologna University and studied in a 1.5 T GE Signa Horizon LX system. The patients satisfied the revised criteria of the International Restless Legs Study Group (IRLSSG) (Allen *et al.*, 2003). Secondary forms of restless legs syndrome were systematically excluded by exploring a detailed history, by neurological and physical evaluation and using laboratory analyses including haemoglobin, iron, ferritin, transferrin, creatinine, urea and liver enzymes. Three patients were subsequently excluded because of evidence of vascular white matter lesions on MRI. The 23 patients studied comprised nine males and 14 females who had a mean $\pm$ SD age of 51 ± 9 years, age at onset of 42 ± 13 years, disease duration of 9 ± 8 years and an IRLSSG rating scale (Walters *et al.*, 2003) score of 22 ± 6. Fourteen patients had never had therapy, and nine patients receiving dopaminergic therapy (pramipexole 0.18 mg, 1 capsule per day for at least 6 months) were free from drugs for at least 2 weeks before the scan. The patients did not take any other drugs at the time of examination or previously. Nine patients had a family history of restless legs syndrome. Brain magnetic resonance studies were carried out during the early afternoon, and only four patients reported mild symptoms of restless legs syndrome during the examination. We also studied 19 healthy control subjects (age 46 ± 13 years; 11 males and eight females). All control subjects were interviewed to exclude symptoms suggesting restless legs syndrome and other neurological disorders or a family history of restless legs syndrome. Twelve of 23 patients were involved in a previous study (Rizzo *et al.*, 2012) that used a different methodological approach for VBM and DTI analysis. All subjects gave written informed consent for their inclusion in the study, which was approved by the institutional review board. None of the patients and controls showed evidence of grey or white matter morphological or signal intensity abnormalities on conventional magnetic resonance (axial FLAIR T_2-weighted, coronal fast-spin echo T_2-weighted, 3D axial T_1-weighted sequences).

Statistical analysis

The difference in sex distribution between patients and healthy controls was evaluated with chi-square test. A Student *t*-test was performed for comparison of the age at examination. *P*-values < 0.05 were accepted as statistically significant.

1H-magnetic resonance prectroscopy study

High-resolution T_1-weighted axial volumetric images were acquired using a fast spoiled gradient-recalled echo sequence and reconstructed in sagittal and coronal plans to better localize the volume of interest. Single voxel 1H-MRS spectra were acquired using a point resolved spectroscopy sequence. The water signal was suppressed by a chemical shift selective sequence. A spectrum at short echo time (echo time = 35 ms; repetition time = 4 s; number of acquisitions = 128) was acquired in the medial region of the thalamus (volume 4.0–5.0 cm^3, with the inferior–superior dimension = 12 mm in all cases) (Fig. 1A). Peak areas of *N*-acetyl-aspartate, creatine–phosphocreatine, choline-containing compounds and myo-inositol were calculated using LCModel, a fitting program that analyses spectra as a linear combination of complete model spectra of metabolite solutions *in vitro* (Provencher, 1993). Peak integral values were expressed relative to creatine–phosphocreatine.

In addition, unsuppressed water spectra were acquired at the same voxel locations using the same sequence, and metabolite concentrations were estimated using tissue water as an absolute internal concentration reference. To compensate for the variable amount of the CSF included in the volume of interest, to avoid an artifactual modification of reported metabolite levels, especially in the presence of atrophy, the concentrations of the metabolites were corrected for the proportion of the CSF volume within the volume of interest obtained using the FMRIB software library (www.fmrib.ox.ac.uk/fsl) FAST segmentation program (Gasparovic *et al.*, 2006). For this purpose, the volume of interest localizations were registered to the segmented tissue maps as obtained by the FAST routine for each subject,
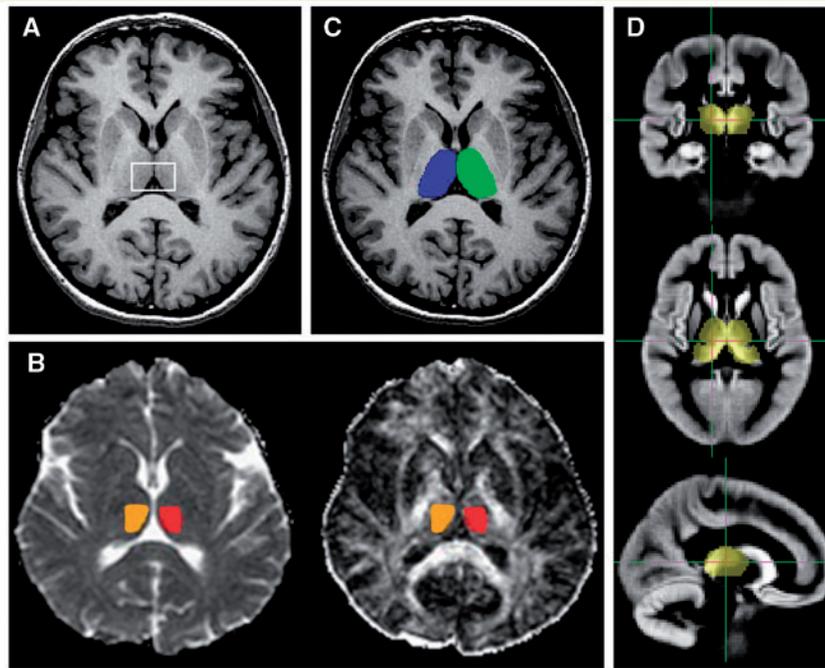
Figure 1 (**A**) Localization of medial thalamic volume of interest for ^{1}H-MRS study. (**B**) Region of interest for DTI analysis on mean diffusivity (*left*) and fractional anisotropy (*right*) maps. (**C**) FIRST segmentation of the thalamus for the volumetric and shape analysis. (**D**) Templates created by FSL-VBM. VBM analysis was only performed within a region defined by the FSL thalamus-maxprob-thr25 template (in yellow).

and the portion of CSF included in the MRS voxel was determined to correct the metabolite concentrations accounting for the fraction of extracellular water present. Concentrations were reported in millmoles. The exclusion criterion for metabolite evaluation was an LCModel estimated fitting error $>20\%$, this being a reliable indicator of poor quality spectra.

Statistical analysis

Parametric tests were used for comparison of MRS data because Kolmogorov–Smirnov testing showed that the variables were normally distributed. Analysis of covariance (ANCOVA) was used to evaluate differences between the two subject groups, with age, gender and therapy as potential covariates. Covariates were introduced by a backward method until the most significant adjusted model was obtained. Only P-values < 0.05 were accepted as statistically significant, after Bonferroni correction for the seven tests of the MRS data. To fully investigate the effect of demographic and clinical parameters (gender, age at onset, disease duration, therapy, positive family history and IRLSSG rating scale score) on patient data, we used a multiple regression with a backward stepwise method to obtain best model in which all included variables had a $P < 0.05$.

Diffusion tensor imaging analysis

Axial DTI images were obtained (4-mm slice thickness without inter-slice gap) using a single-shot spin echo–echo planar sequence with echo time = 89.2 ms; repetition time = 10 s; 32 cm^2 field of view, in-plane resolution = 192 × 192 and number of excitations = 2. One T$_2$-weighted scan without diffusion gradients was acquired and six with direction-encoding gradients at strengths corresponding to b-value 900 s/mm^2.

DTI processing was performed using the FMRIB software library (FSL; www.fmrib.ox.ac.uk/fsl). DTI-echo planar images were registered using the image registration software FLIRT. Distortions due to gradient-induced eddy currents were corrected slice-wise, restricting degrees of freedom to translation, scaling and shearing along the phase encoding direction (Haselgrove and Moore, 1996). Possible head movements were corrected by registering each volume to the first, allowing only translation and rotation. Parameter maps for mean diffusivity and fractional anisotropy were determined voxel-wise using the program DTIFIT. Regions of interest at level of left and right medial thalamus were delineated on T$_2$-weighted images in three consecutive slices, to outline the same parenchyma studied by spectroscopy, and were then transposed to mean diffusivity and fractional anisotropy maps (Fig. 1B).

Statistical analysis

For comparison of DTI, data parametric tests were used as Kolmogorov–Smirnov testing, which showed that the variables were normally distributed. An ANCOVA was used to evaluate differences between the two subject groups, with age, gender and therapy as potential covariates. Covariates were introduced by a backward method until the most significant adjusted model was obtained. Only P-values < 0.05 were accepted as statistically significant, after Bonferroni correction for the four tests of the DTI data. To fully investigate the effect of demographic and clinical parameters (gender, age at onset, disease duration, therapy, positive family history and IRLSSG rating scale score) on patient data, we used a multiple regression with a backward stepwise method to obtain best model in which all included variables had a $P < 0.05$.

Volumetric analysis

Automatic segmentation of the volumetric image was performed using FMRIB's Integrated Registration and Segmentation Tool (FIRST) to delineate the left and right thalami (Fig. 1C).

Statistical analysis

Left and right thalamic volumes were calculated and corrected for total intracranial volume because initial testing indicated that intracranial volumes were correlated. For comparison of volumetric data, parametric tests were used as Kolmogorov–Smirnov testing, which showed that the variables were normally distributed. An ANCOVA was used to evaluate differences between the two subject groups, with age, gender and therapy as potential covariates. Covariates were introduced by a backward method until the most significant adjusted model was obtained. Only P-values < 0.05 were accepted as statistically significant after Bonferroni correction for the two tests of the volume data. To fully investigate the effect of demographic and clinical parameters (gender, age at onset, disease duration, therapy, positive family history and IRLSSG rating scale score) on thalamic volumes, we used a multiple regression with a backward stepwise method to obtain best model in which all included variables had a $P < 0.05$.

Voxel-based morphometry analysis

A T_1-weighted axial volumetric image was acquired using a fast spoiled gradient echo sequence (inversion time $= 600$ ms; echo time $= 5.1$ ms; repetition time $= 12.5$ ms; 25.6 cm^2 field of view, 1-mm slice thickness; in-plane resolution $= 256 \times 256$).

Structural data were analysed using FSL-VBM (www.fmrib.ox.ac.uk/fsl) software tools. Structural images were brain extracted using Brain Extraction Tool (BET) (Smith, 2002). Next, tissue-type segmentation was carried out using FAST4 (Zhang *et al.*, 2001). The resulting grey matter partial volume images were then aligned to Montreal Neurological Institute MNI152 standard space using the affine registration tool FLIRT (Jenkinson *et al.*, 2002), followed by non-linear registration using FNIRT. The resulting images were averaged to create a study-specific template, to which the native grey matter images were then non-linearly re-registered. Registered partial volume images were then modulated by the Jacobian of the warp field. The modulated volume images were then smoothed with an isotropic Gaussian kernel (full width at half-maximum 7.1 mm).

Statistical analysis

Group comparison of patients and controls was performed voxel-wise based on an ANCOVA model, including sex, age and therapy as covariates. Testing was only performed within a region defined by a template to include the thalamus. The thalamic template was based on the FSL thalamus-maxprob-thr25 template, excluding voxels for which the mean-adjusted grey matter fraction for our data was $<3\%$ (Fig. 1D). We considered as significant voxels for which $P < 0.05$ corrected for multiple comparisons using the family-wise error rate. A threshold-free cluster enhancement was applied. For patients, we performed linear regression voxel-wise on the thalamic tissue volumes against subjects' IRLSSG scores, age at onset and disease durations ($P < 0.05$ corrected). The analysis was also repeated without covariates and using a $P < 0.001$ uncorrected to rule out any possible false negative results.

Shape analysis

For each of the left and right thalami identified by FIRST (see 'Volumetric analysis' section), a comparable list of boundary vertices was created.

Statistical analysis

An ANCOVA was used to evaluate differences among two groups, using age, gender and therapy as covariates. Only P-values < 0.05 were accepted as statistically significant, adjusted using the False Discovery Rate method. To fully investigate the effect of demographic and clinical parameters on thalamic volumes, we performed a multiple regression with gender, age at onset, disease duration, therapy, positive family history and IRLSSG rating scale score as covariates. The analysis was also repeated without covariates and using $P < 0.001$ uncorrected to rule out any possible false negative results.

Results

There was no difference in sex distribution and age at examination between groups. The ^{1}H-MRS study revealed a significantly reduced N-acetylaspartate/creatine-phosphocreatine ratio and N-acetylaspartate concentration at the level of the medial thalamus in patients with restless legs syndrome compared with healthy controls (Table 1 and Figs 2 and 3), whereas choline-containing compounds/creatine–phosphocreatine, myo-inositol/creatine–phosphocreatine ratios, and choline containing compounds, creatine–phosphocreatine and myo-inositol concentrations were similar in patients and control subjects. The results were significant whether or not covariates (age, gender and therapy) were considered, being slightly better without covariates (Table 1). Regression analysis disclosed a significant association between N-acetylaspartate concentration and the family history ($\beta = -0.49$; $P = 0.018$): lower N-acetylaspartate concentrations were associated with a positive family history of restless legs syndrome. No effect was disclosed for any other MRS variable.

In contrast, the DTI analysis, volumetric analysis (Table 2), VBM and shape analysis (Fig. 4) showed neither difference between patients and controls nor correlation between magnetic resonance parameters and clinical data in the subjects with restless legs syndrome. Removing covariates from the group comparison did not yield a significant result, nor did lower the significance threshold (to 0.001 uncorrected) in the VBM and shape analyses.

The four patients complaining of symptoms during the magnetic resonance examination presented results no different from the other patients (data not shown). Although the patients receiving dopaminergic therapy were free from drugs for at least 2 weeks before scan, we performed a further separate analysis comparing patients with (age: 56 ± 7 years; male/female: 5/4) and without treatment (age: 49 ± 10 years; male/female: 4/10) using ANCOVA (with gender and age as covariates) to fully exclude a pharmacological effect on any of the MRS variables. In patients with restless legs syndrome previously treated with dopaminergic drugs, thalamic N-acetylaspartate/creatine–phosphocreatine (1.19 ± 0.19) was similar to that found in patients never treated (1.25 ± 0.13; uncorrected $P = 0.24$, corrected $P = 1$); likewise, N-acetylaspartate concentrations were no different (8.7 ± 1.4 mM compared with 9.3 ± 0.9 mM in untreated patients; uncorrected $P = 0.17$, corrected $P = 1$). Similarly, no changes were detected between these two subgroups of patients for any other magnetic resonance variables evaluated (data not shown).

Table 1 Results of medial thalamic H^1-MRS study

	Controls (n = 19)		Patients with restless legs syndrome (n = 23)		Uncorrected P^a	Corrected P^a
	Mean	±SD	Mean	±SD		
NAA/Cr	1.39	0.11	1.23	0.16	0.0007	**0.005**
Cho/Cr	0.31	0.04	0.29	0.03	0.096	0.67
mI/Cr	0.80	0.16	0.79	0.15	0.91	1
[NAA]	10.36	1.12	9.05	1.14	0.0007	**0.005**
[Cr]	7.66	0.85	7.42	0.75	0.33	1
[Cho]	2.29	0.20	2.14	0.21	0.025	0.18
[mI]	5.86	0.70	5.89	1.27	0.91	1

[a] ANCOVA using age, gender and therapy as covariates (P-values < 0.05 corrected for multiple comparisons were considered significant, and are reported in bold).

[] = absolute concentrations reported in millimolar; Cho = choline-containing compounds; Cr = creatine-phosphocreatine; mI = myo-inositol; NAA = N-acetylaspartate.
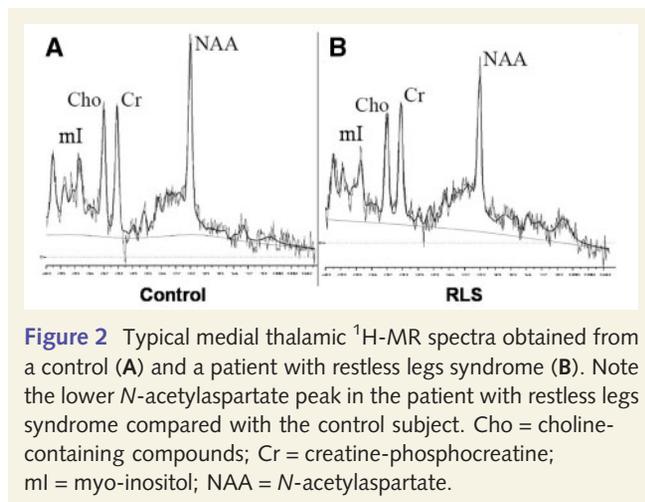

Figure 2 Typical medial thalamic ^{1}H-MR spectra obtained from a control (**A**) and a patient with restless legs syndrome (**B**). Note the lower N-acetylaspartate peak in the patient with restless legs syndrome compared with the control subject. Cho = choline-containing compounds; Cr = creatine-phosphocreatine; mI = myo-inositol; NAA = N-acetylaspartate.

Discussion

Using ^{1}H-MRS, we detected a reduction in the neuronal marker N-acetylaspartate in the medial region of the thalamus of patients with restless legs syndrome. This alteration was more evident when the patients had a positive family history of restless legs syndrome. Given that no structural changes were detected by DTI analysis, volumetric analysis, VBM and shape analysis, the ^{1}H-MRS thalamic alteration likely reflects either a neuronal loss too small to be detected by the other methods used or a neuronal dysfunction. The latter case is supported by the normal content of myo-inositol/creatine–phosphocreatine and myo-inositol concentration in the thalamus of patients with restless legs syndrome. In neurodegenerative disorders, neuronal loss is in general associated with glial activation, responsible for the increase in the glial marker myo-inositol (Brand et al., 1993) as shown in the thalamus of patients with Creutzfeldt–Jakob disease (Lodi et al., 2009), where reduced N-acetylaspartate, expression of neuronal loss, was associated with increased myo-inositol expression of increased glial cell content.

N-acetylaspartate is synthesized in neurons and while its role is not fully elucidated, it is currently considered a marker for neuronal health, viability and number (Moffett et al., 2007). A reduction in N-acetylaspartate levels reflects neuronal or axonal loss in several neurological disorders (Moffett et al., 2007). On the other hand, studies in animals have also documented lower N-acetylaspartate concentrations in a contest of neuronal dysfunction without cell loss (Dautry et al., 2000; Demougeot et al., 2001). The role of N-acetylaspartate as a marker of neuronal function in humans is also supported by the reversibility of the local N-acetylaspartate reduction observed in patients with multiple sclerosis (Arnold et al., 1990), traumatic brain injury (Signoretti et al., 2001), stroke (Walker et al., 2004) and epilepsy (Briellmann et al., 2005).

The thalamus is a crucial region for a number of brain networks including the limbic system. The limbic structures are important in different cerebral functions including pain processing, being part of the so-called medial nociceptive system. This system projects through medial and intralaminar nuclei of the thalamus to several cortical and limbic regions: frontal and insular cortices and anterior cingulate gyrus (Price, 2000). The medial nociceptive system mediates affective–motivational aspects of pain such as emotional reactions, arousal and attention to the stimulus, as well as the drive to escape from the noxious stimuli (Treede et al., 1999; Price, 2000). An activation of these brain structures during pain perception has been confirmed by PET and functional MRI studies (Apkarian et al., 2005). H^2[^{15}O] PET (San Pedro et al., 1998) and functional MRI (Bucher et al., 1997; Astrakas et al., 2008) studies also found activation of some of these structures in patients with restless legs syndrome. A PET study with [^{11}C]diprenorphine, a non-selective opioid receptor radioligand, disclosed regional negative correlations between ligand binding and restless legs syndrome severity in areas serving the medial pain system (medial thalamus, amygdala, caudate nucleus, anterior cingulate gyrus, insular cortex and orbitofrontal cortex) (von Spiczak et al., 2005). Another PET study that used the high-affinity D2 receptors radioligand [^{11}C]FLB 457 reported a higher thalamic-binding potential in patients with restless legs syndrome than in controls, at the level of the medial and posterior portions of thalamus, other than at the level of limbic and associative part of striatum, anterior
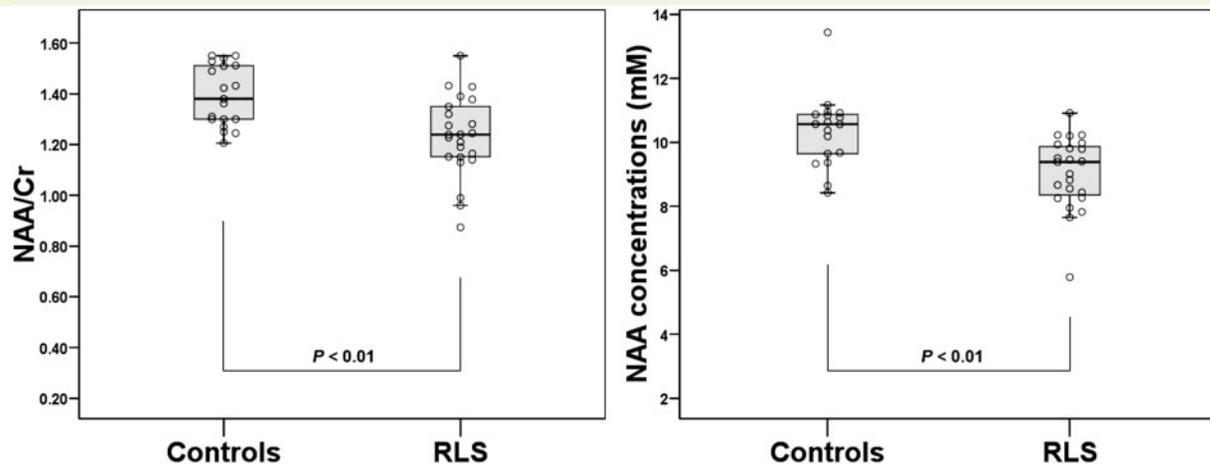
Figure 3 Medial thalamic *N*-acetylaspartate (NAA) to creatine-phosphocreatine (Cr) ratios and absolute *N*-acetylaspartate concentrations in the 23 patients with restless legs syndrome and the 19 controls. Scatter plots of individual values are superimposed on box-plots indicating the median, quartiles and extreme values (within 1.5 times the interquartile range from the upper or lower quartile). Reported *P*-values are corrected for multiple comparisons.

Table 2 Results of medial thalamic DTI and volumetric studies

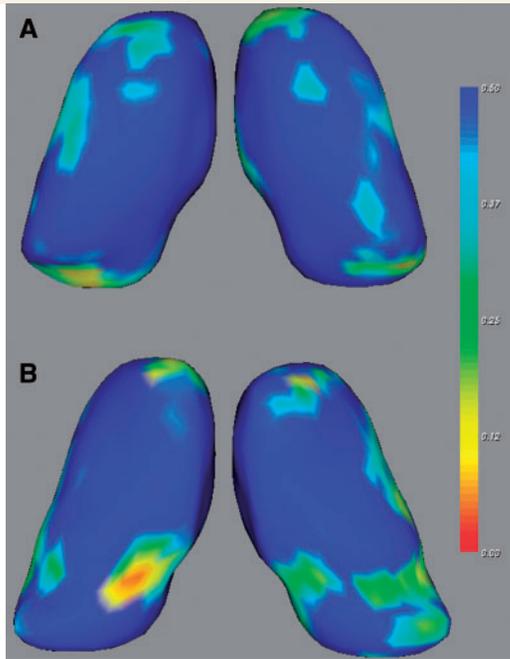|	Controls (n = 19)		Patients with restless legs syndrome (n = 23)		Uncorrected P^a	Corrected P^a
	Mean	±SD	Mean	±SD		
DTI analysis						
L thalamus MD	0.77	0.03	0.77	0.05	0.62	1
L thalamus FA	0.31	0.02	0.31	0.03	0.55	1
R thalamus MD	0.78	0.03	0.78	0.04	0.77	1
R thalamus FA	0.33	0.03	0.34	0.04	0.37	1
Volumetric analysis						
L thalamus VOL	1 1293	679	1 1078	1045	0.45	0.9
R thalamus VOL	1 0912	724	1 0671	1111	0.43	0.86

[a]ANCOVA using age, gender and therapy as covariates (*P*-values < 0.05 corrected for multiple comparisons were considered significant).
FA = fractional anisotropy; MD = mean diffusivity (x10^{-3}mm^2/s); VOL = volume (cm^3) adjusted for total intracranial volume; L = left; R = right.

cingulate cortex and insulae, confirming an involvement of the medial pain system (Cervenka *et al.*, 2006). Neurophysiological studies have suggested a functional impairment of central somatosensory processing in restless legs syndrome (Schattschneider *et al.*, 2004; Stiasny-Kolster *et al.*, 2004; Tyvaert *et al.*, 2009; Bachmann *et al.*, 2010; Edwards *et al.*, 2011). Some of these focused on pain processing in patients with idiopathic restless legs syndrome who exhibited static mechanical hyperalgesia relating to hyperalgesia to blunt pressure, hyperalgesia to pinprick stimuli and vibratory hyperaesthesia (Stiasny-Kolster *et al.*, 2004; Bachmann *et al.*, 2010). The increased ratings of pinprick pain in untreated patients with restless legs syndrome was more pronounced in the lower limb and reversed by long-term dopaminergic treatment (Stiasny-Kolster *et al.*, 2004). A recent study also revealed a temporal summation of heat pain, which may reflect aberrant CNS facilitation of pain transmission (Edwards *et al.*, 2011).

Interestingly, a post-mortem immunohistochemistry study identified diminished numbers of β-endorphin- and met-enkephalin-positive cells in the thalamus of patients with restless legs syndrome, supporting the presence of altered central processing of pain and implication of the endogenous opioid system in the pathogenesis of restless legs syndrome (Walters *et al.*, 2009). Furthermore, a dysfunction in central nociceptive structures is consistent with the sensory descriptors of the sensations in restless legs syndrome, similar to those in neuropathic pain (Karroum *et al.*, 2012), but more focused on emotionally unpleasant content rather than an actual pain.

Our MRS data confirm a thalamic neuronal dysfunction in patients with restless legs syndrome, presumably not due to degenerative changes as no structural changes were detected in the same thalamic area by the other techniques employed. This is consistent with most structural magnetic resonance studies using DTI or VBM to look for subtle morphological changes that show no changes in the medial thalamus of patients with restless legs syndrome (Hornyak *et al.*, 2007; Unrath *et al.*, 2007; Celle *et al.*, 2010b; Comley *et al.*, 2012; Rizzo *et al.*, 2012). One DTI study revealed reduced fractional anisotropy at the level of the posterior ventral lateral nucleus, but only on the right side (Unrath *et al.*,

Figure 4 Results of shape analysis: No significant differences between patients with restless legs syndrome and healthy controls were found. (**A**) Superior view and (**B**) inferior view. Colour bars represent uncorrected *P*-values for the comparison of the two groups from analysis of covariance analysis of the boundary vertices of the thalamus created by the software.

2008); in contrast, a more recent study found no significant results (Rizzo *et al.*, 2012). Among VBM studies, only one revealed a thalamic change, specifically an increase in grey matter density in the pulvinar of patients with restless legs syndrome (Etgen *et al.*, 2005). This finding was interpreted as secondary to chronic increase in afferent input of behaviourally relevant information (Etgen *et al.*, 2005), but it could have been also explained as a compensation of an underlined dysfunction. Similarly, the spectroscopic alterations disclosed in our study could be an epiphenomenon of chronic discomfort perception or sleep disruption as suggested by the effect of sleep deprivation in healthy women (Smith *et al.*, 2007). On the contrary, the significant association between lower *N*-acetylaspartate concentrations and the presence of family history of restless legs syndrome may support the interpretation of the thalamic changes as a pathophysiological marker of disease rather than a secondary phenomenon, at least for the familial forms. Accordingly, we may speculate that the involvement of the medial portion of the thalamus could have a primary role, because its function is modulated by dopaminergic afferents. Indeed, an extensive mesothalamic and nigrothalamic system originates as collaterals from A8–A9–A10 dopaminergic neurons (Freeman *et al.*, 2001). Thus, dopaminergic axons directly innervate thalamic components of several parallel, functionally unique, basal ganglia-thalamocortical loops as follows: motor (ventrolateral), 'prefrontal' (parvocellular ventroanterior) and 'limbic' (mediodorsal; mean diffusivity) in non-human primates and humans (Rye, 2004). Accordingly, the thalamic metabolic

alteration that we have found may reflect an impairment of medial pain system secondary to a dopaminergic dysfunction and leading to an abnormal affective-motivational sensory motor processing of the sensory inputs. This could also happen in parallel with a dysfunction of other dopaminergic pathways such as the diencephalospinal pathway projecting from A11 area to spinal cord (Clemens *et al.*, 2006) in a context of a multilevel demodulation of pain stimuli perception. From this point of view, the motor symptoms of restless legs syndrome could derive from a disinhibition of an innate behaviour to escape from an indefinite painful noxa.

Other interpretations of our data may be possible due to the many functions of the limbic system and specifically of the medial thalamus. The thalamic spectroscopic alterations could be secondary to the sleep impairment itself, considering the role of the medial thalamus in sleep regulation (Montagna, 2011). Furthermore, the medial thalamus also has a role in the cognitive and affective field (Tekin and Cummings, 2002; Drevets *et al.*, 2008; Clark *et al.*, 2009; De Witte *et al.*, 2011), and patients with restless legs syndrome are more prone to developing depressive and anxiety disorders (Winkelmann *et al.*, 2005; Cho *et al.*, 2009; Hornyak, 2010; Celle *et al.*, 2010a) and mild cognitive deficits (Pearson *et al.*, 2006; Gamaldo *et al.*, 2008; Fulda *et al.*, 2010; Celle *et al.*, 2010a). Even if the actual role of the sleep impairment in the cognitive and mood disorders in restless legs syndrome is not fully elucidated, the medial thalamus may be one of the structures whose alteration, primary or secondary, underlie these symptoms.

Possible limitations of this study include (i) 12 of the 23 patients of this study were included in a previous VBM study (Rizzo *et al.*, 2012) of grey matter volumes and DTI parameters. This study also includes such analyses but considers only the thalamus, not the whole brain, to increase sensitivity. The additional measurements and analyses (of thalamic ^{1}H-MRS, volume and shape) have not previously been conducted in studies of patients with restless legs syndrome; (ii) patients with restless legs syndrome were evaluated only once in the afternoon, so circadian changes could not be investigated; (iii) some patients had been treated with dopaminergic drugs. However, the impact on ^{1}H-MRS findings was probably marginal for several reasons: only 9 of 23 patients were treated; therapy was stopped 2 weeks before scan; the statistical models (ANCOVA and multiple regression) including therapy as a covariate did not disclose any effect on thalamic *N*-acetylaspartate and the direct comparison between patients with and without treatment using ANCOVA did not show significant differences; and (iv) there is a mild gender imbalance between patients with restless legs syndrome and controls. However, the chi-square test excluded a significant difference between the two groups, and we used the gender as a covariate in the ANCOVA and the multiple regression analyses without altering the results.

In conclusion, our findings demonstrate an abnormal medial thalamus metabolism in idiopathic patients with restless legs syndrome, suggesting a role for the dysfunction in the limbic system in the pathophysiology of restless legs syndrome. Further investigations will be useful to better understand the exact impact of this dysfunction on the phenomenology of restless legs syndrome.

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Funding

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