RLS is a common sensorimotor disorder in which sensory unease evokes motor restlessness of the lower limbs. The symptoms, SLD and PLMs, fluctuate in severity with the diurnal cycle, worsening during the night-time hours. RLS encompasses eRLS, which is mainly idiopathic, and late-onset RLS, which is usually a secondary form. eRLS affects younger patients with a positive family history and has been correlated with a gene variant (btbd9) that is associated with iron content in the midbrain. Histopathologic studies in idiopathic RLS have shown decreased iron staining in the substantia nigra and minimal transferrin-receptor staining in neuromelanin cells. MR imaging studies by using T2 relaxometry metrics have shown a lower iron content of the substantia nigra in eRLS and late-onset RLS. With VBM, the brain volume has been assessed in patients with RLS, with conflicting findings. Small sample size, use of different techniques, inclusion of patients under medication, and lack of distinction between eRLS and late-onset RLS were probably responsible for the inconsistency in observations.

fMRI studies have been conducted in patients with RLS with undefined disease onset and late onset. fMRI during episodes of combined sensory and motor symptoms has revealed activity in the cerebellum, red nucleus, and thalamus. Patients with late-onset RLS performing regular dorsiflexion and plantar flexion of the feet in hours of daylight demonstrated greater activation of the dorsolateral prefrontal cortex of the left middle gyrus and inferior frontal gyrus and, marginally, of the cingulate gyrus. Although VBM, T2 relaxometry, and fMRI have all been used in the study of patients with RLS, the 3 techniques have never been applied simultaneously in the same patient population. T2 relaxometry detects ultrastructural tissue changes by decline of the transverse component of the magnetization due to irreversible dephasing; it is influenced by water content, cerebral blood flow, and iron deposition. VBM is an automated technique which, by using voxelwise statistical analysis, detects brain-volume differences with no a priori assumptions about their location. fMRI evaluates brain function by detection of hemodynamic changes related to brain activation. Evaluation of the same eRLS population for brain volume changes, brain iron content, and brain activation during episodes of RLS symptoms...
could provide improved insight into the pathophysiology of the disease. In this study, patients with unmedicated eRLS were assessed by brain MR imaging by using VBM and T2 relaxometry metrics to analyze brain volume and T2 relaxation time, respectively. fMRI was performed in the night hours during episodes of exacerbation of symptoms.

Materials and Methods
The study included 11 right-handed patients with idiopathic eRLS who had never been treated with dopaminergic agents (9 women, 2 men; range, 48–70 years of age; mean 55.3 ± 8.4 years; mean disease duration, 17.5 ± 14.05 years) and 11 sex- and age-matched right-handed control subjects (9 women, 2 men; range, 42–73 years of age; mean, 56.09 ± 9.6 years) with no symptoms of RLS. All the study patients with RLS had early disease onset, with symptoms starting before 45 years of age. Lateralization of the symptoms was not reported by any of the patients. The patients underwent a detailed history survey, physical examination, laboratory tests (routine biochemistry including thyroid function, vitamins, and electrolytes) and nerve-conduction studies (electromyography). Their condition was chemistry including thyroid function, vitamins, and electrolytes) and history survey, physical examination, laboratory tests (routine bio-

The VBM method by using the unified segmentation approach was implemented by using the T1-weighted images. A scanner-specific template of GM, WM, and CSF compartments was constructed on measurements of 24 healthy subjects (12 women, 12 men; mean age, 58.9 ± 10.6 years). VBM involved simultaneous normalization of all images according to the scanner-specific template: correction for intensity inhomogeneity and segmentation of GM, WM, and CSF compartments. Morphologic differences between the patients with eRLS and control subjects were estimated by using an independent-samples t test at the voxel level. Comparison between patients and control subjects was made for 2 different contrasts corresponding to increase (eRLS > controls) or decrease (eRLS < controls) of brain volume in the GM and WM compartments. The statistical parametric maps were thresholded by using a false discovery rate at P < .05, corrected for multiple comparisons.

T2 relaxometry images were processed off-line by using Matlab 7.6 (MathWorks, Natick, Massachusetts). A monoexponential function of the form $S(TE) = S_0 \cdot \exp (-TE/T2) + C$ was assumed to describe the signal-intensity decay with TE, where $S(TE)$ is the signal intensity at TE, $S_0$ is the signal intensity amplitude at TE = 0, and C is a constant offset parameter added to compensate for background noise bias. A T2 map was produced by a pixel-by-pixel fitting procedure, by using the Levenberg-Marquardt optimization method. The T2 relaxation time of the putamen, caudate nucleus, globus pallidus external and internal substantia nigra pars compacta and reticulata, STN, red nucleus, and locus coeruleus was measured by using the region-of-interest function by a method previously described.

To check for inter-rater agreement, a second researcher (M.I.A.) performed T2 relaxation time measurements and used the Pearson product-moment correlation coefficient. A P value <.05 was considered statistically significant. Differences in the T2 relaxation times between patients and control subjects were evaluated by using the unpaired 2-tailed Student t test.

fMRI data preprocessing was performed by using Matlab 7.6 (MathWorks) and SPM software (SPM5, Wellcome Department of Cognitive Neurology, London, UK). EPI images were realigned both spatially to minimize residual head movement and temporally to ensure that the data from any given section were sampled at the same time. The images were then coregistered with the high-resolution anatomic image, normalized with the MNI template, and smoothed by using a Gaussian kernel of 8 × 8 × 8 mm. The monitored PLMs of each patient were modeled in the first-level analysis. Second-level random-effects analysis was used to determine activated areas within the group, and an activation map was generated. Motor-activated regions were derived at a significance level of uncorrected P < .001, with an extent threshold of 10 voxels. The regions (clusters) that were
activated and their laterality were identified registering the Talairach coordinates of the SPM maps to the WFU PickAtlas toolbox, an automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI datasets. (WFU PickAtlas, Version 2.0, http://dbic.dartmouth.edu/pipermail/mrusers/attachments/20050923/ d4ae1062/attachment.pdf).19 Potential atlas-induced errors were checked by visual estimation on the T1 MNI template of the regions of activated regions and the corresponding coordinates of the SPM maps to the WFU PickAtlas toolbox, an automatic method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI datasets. (WFU PickAtlas, Version 2.0, http://dbic.dartmouth.edu/pipermail/mrusers/attachments/20050923/d4ae1062/attachment.pdf).19 Potential atlas-induced errors were checked by visual estimation on the T1 MNI template of the regions of activated regions and the corresponding coordinates of the SPM maps to the WFU PickAtlas toolbox, an automatic method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI datasets. (WFU PickAtlas, Version 2.0, http://dbic.dartmouth.edu/pipermail/mrusers/attachments/20050923/d4ae1062/attachment.pdf).

### Results

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

The Pearson correlation coefficient of the raters’ T2 measurements was 0.95, indicating strong inter-rater agreement. The T2 relaxation time was significantly lower in patients with eRLS than in the control subjects in the right STN (patients, 6.15; controls, 6.59; P < .001). Postmortem studies have demonstrated low iron content in the substantia nigra of patients with eRLS who received treatment with dopaminergic drugs.4,6,27 In the present study, by using a spin-echo technique, the T2 relaxation time of the substantia nigra pars compacta and reticulata was evaluated separately, and no significant difference was found between the patients with untreated eRLS and control subjects. Gradient-echo sequences are more sensitive in detecting iron; thus, measurement of T2* relaxation time might be more efficient in detecting differences in tissue iron content.28 Nevertheless, in practice, T2* and T2’ are more prone to artifacts and T2* is more dependent on factors unrelated to iron, such as susceptibility artifacts and the BOLD effect.29 Treatment-induced changes may account for the differences observed in the other studies. Indeed experimental studies have demonstrated that treatment with dopaminergic drugs may affect neuronal viability and iron content.30 The present study is the first to demonstrate decreased T2 relaxation times, suggestive of increased iron content in the globus pallidus internal and the STN of patients with RLS. Secondary RLS has been associated with low serum ferritin levels.31 This is not in contradiction with the results of the current study because ferritin does not cross the blood-brain barrier; therefore, its serum levels are not a good index of brain iron burden.18,32

Although iron is critical in brain metabolism, increased iron deposition occurs in brain areas undergoing degeneration.9,17,33 According to the currently accepted model of functional neuroanatomy, the globus pallidus external, through excitatory D2 receptors, receives GABAergic inhibitory projections from the striatum, and the STN receives GABAergic

### Discussion

The major findings of this study in patients with eRLS were absence of changes in brain volume, increased iron content in the right globus pallidus internal and the STN, and observation on fMRI of increased activation in the right hemisphere of the striatum, the parietal lobule, and the dorsolateral prefrontal cortex; and in the left hemisphere of the precentral gyrus, the postcentral gyrus, the pars opercularis, the thalamus, and the ventral anterior cingulum.

VBM techniques have been previously applied in patients with RLS with conflicting results.7-9,20,21 An increase in the volume of the thalamus was reported in 1 study performed on 2 independent RLS series,8 and a decrease in the volume of the primary somatosensory and primary motor cortex was reported in a second study.7 GM volume changes have been demonstrated in patients treated with medications affecting the dopamine system; thus, brain volume changes in patients with RLS under medication may represent a secondary pharmacologic effect on brain plasticity.21-24 Marginally significant GM volume changes have been reported in only 1 study of unmedicated patients.9 In agreement with the present study, 2 earlier studies performed on unmedicated patients with RLS revealed no significant volume change in comparison with control subjects.20,21

There is evidence that brain iron content plays an important role in the pathophysiology of RLS.25,26 Postmortem studies have demonstrated low iron content in the substantia nigra of patients with eRLS who received treatment with dopaminergic drugs.4,6,27 In the present study, by using a spin-echo technique, the T2 relaxation time of the substantia nigra pars compacta and reticulata was evaluated separately, and no significant difference was found between the patients with untreated eRLS and control subjects. Gradient-echo sequences are more sensitive in detecting iron; thus, measurement of T2* relaxation time might be more efficient in detecting differences in tissue iron content.28 Nevertheless, in practice, T2* and T2’ are more prone to artifacts and T2* is more dependent on factors unrelated to iron, such as susceptibility artifacts and the BOLD effect.29 Treatment-induced changes may account for the differences observed in the other studies. Indeed experimental studies have demonstrated that treatment with dopaminergic drugs may affect neuronal viability and iron content.30 The present study is the first to demonstrate decreased T2 relaxation times, suggestive of increased iron content in the globus pallidus internal and the STN of patients with RLS. Secondary RLS has been associated with low serum ferritin levels.31 This is not in contradiction with the results of the current study because ferritin does not cross the blood-brain barrier; therefore, its serum levels are not a good index of brain iron burden.18,32

Although iron is critical in brain metabolism, increased iron deposition occurs in brain areas undergoing degeneration.9,17,33 According to the currently accepted model of functional neuroanatomy, the globus pallidus external, through excitatory D2 receptors, receives GABAergic inhibitory projections from the striatum, and the STN receives GABAergic
inhibitory projections from the globus pallidus external and strong excitatory glutamatergic afferents from the motor cortex. Hypofunctioning in brain dopamine signaling is currently accepted as a characteristic in patients with RLS. Dopamine is a catecholamine neurotransmitter exhibiting a circadian rhythm of signaling with a nadir in the night hours. A hypothetic explanation is that patients with RLS may present with night hypofunctioning of the dopamine signaling in the basal ganglia circuitry, which may induce subnormal activation of the D2 receptors of the striatum, resulting in increased activation of the latter. An increase of the GABAergic inhibitory projection from the striatum to the
The basal ganglia may be involved in the pathophysiology of restless legs syndrome (RLS). Hyperfunctioning of the basal ganglia can lead to increased iron content in the globus pallidus, which may be associated with restless motor symptoms. In patients with Parkinson disease, a related movement disorder, hyperfunctioning of the basal ganglia may result in increased iron content in the basal ganglia, leading to iron accumulation, which may contribute to the development of RLS symptoms.

Conclusions
This study has demonstrated that eRLS is not associated with brain-volume changes but with increased iron content in the globus pallidus internal and STN, suggestive of a dysfunction of the basal ganglia circuitry. The observed activation of the striatofrontolimbic circuitry may indicate the neurofunctional substrate mediating the repetitive compulsive movements seen in RLS.

References
1. Garcia-Borreguero D. Time to REST: epidemiology and burden. Eur J Neurol 2006;13:15–20
2. Paulus W, Dowling P, Rijnman R, et al. Update of the pathophysiology of the restless-legs syndrome. Mov Disord 2007;22:8431–39
3. Jellen LC, Beard JL, Jones BC. Systems genetics analysis of iron regulation in the brain. Biochimie 2009;91:1255–59
4. Connor JR, Boyer PJ, Menzies SL, et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. Neurology 2003;61:304–09
5. Earley CJ, B Barker P, Horska A, et al. MRI-determined regional brain iron concentrations in early- and late-onset restless legs syndrome. Sleep Med 2006;7:458–61. Epub 2006 Jun 5
6. Astrakas LG, Kontisitits S, Margariti P, et al. T2 relaxation and fMRI of the brain in late-onset restless legs syndrome. Neurology 2008;71:911–16
7. Unrath A, Juengling FD, Schork M, et al. Cortical grey matter alterations in idiopathic restless legs syndrome: an optimized voxel-based morphometry study. Mov Disord 2007;22:1751–56
8. Engen T, Draganski B, Berg C, et al. Bilateral thalamic gray matter changes in patients with restless legs syndrome. Neuroimage 2005;24:1242–47
9. Hornyk M, Abrehds JC, Spiegelhalder K, et al. Voxel-based morphometry in unmedicated patients with restless legs syndrome. Sleep Med 2007;9:22–26
10. Bucher SF, Seelos KC, Oertel WH, et al. Cerebral generators involved in the pathogenesis of the restless legs syndrome. Ann Neurol 1997;41:439–45
11. Trzouchi LC, Astrakas LG, Kontisitits S, et al. Voxel-based morphometry and voxel-based relaxometry in parkinsonian variant of multiple system atrophy. J Neuroimag 2010;20:260–66
12. Astrakas LG, Argyropoulou MI. Shifting from region of interest (ROI) to voxel-based analysis in human brain mapping. Pediatr Radiol 2010;40:1857–67. Epub 2010 May 13
13. Norris DG. Principles of magnetic resonance assessment of brain function. J Magn Reon Imaging 2006;23:794–807
14. Walters AS, LeBrocq C, Dhar A, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. Sleep Med 2003;4:121–32
15. Benes H, Walters AS, Allen RP, et al. Definition of restless legs syndrome, how to diagnose it, and how to differentiate it from RLS mimics. Mov Disord 2007;22:401–08
16. Ashburner J, Friston KJ. Unified segmentation. Neuroimage 2005;26:839–51
17. Kosta P, Argyropoulou MK, Markoula S, et al. MRI evaluation of the basal ganglia size and iron content in patients with Parkinson’s disease. J Neurol 2006;253:26–32
18. Metatratzi A, Argyropoulou M, Kiortitsis DN, et al. T(2) relaxation rate of basal ganglia and cortex in patients with beta-thalassaemia major. Br J Radiol 2001;74:407–10
19. Maldjian JA, Laurienti PJ, Kraft RA, et al. An automated method for neuroanatomically and cytoarchitecturally Atlas-based interrogation of fMRI data sets. Neuroimage 2003;19:1233–39
20. Celle S, Roche F, Feyron R, et al. Lack of specific gray matter alterations in restless legs syndrome in elderly subjects. J Neurol 2012;257:344–48
21. Comley RA, Cervenka S, Palhagen SE, et al. Comparison of gray matter density...
in restless legs syndrome patients and matched controls using voxel-based morphometry. J Neuroimaging 2012;22:28–32.

22. Chakos MH, Lieberman JA, Abur J, et al. Caudate nuclei volumes in schizophrenic patients treated with typical antipsychotics or clozapine. Lancet 1995;18:456–57

23. Scheepers FE, Gespen de Wied CC, Kahn RS. The effect of olanzapine treatment on m-chlorophenylpiperazine-induced hormone release in schizophrenia. J Clin Psychopharmacol 2001;21:575–82

24. Tyvaert L, Houdayer E, Devanne H, et al. Cortical involvement in the sensory and motor symptoms of primary restless legs syndrome. Sleep Med 2009 10:1090–96

25. Clements S, Rye D, Hochman S. Restless legs syndrome revisited: the dopaminergic hypothesis from the spinal cord perspective. Neurology 2006;67:125–30

26. Connor JR, Ponnuru P, Wang XS, et al. Profile of altered brain iron acquisition in restless legs syndrome. Brain 2011;134:959–68. Epub 2011 Mar 11

27. Connor JR, Wang XS, Patton SM, et al. Decreased transferrin receptor expression by neuromelanin cells in restless legs syndrome. Neurology 2004;62:1563–67

28. Rodrigue KM, Haacke EM, Raz N. Differential effects of age and history of hypertension on regional brain volumes and iron. Neuroimage 2011;54:790–59

29. Argyropoulou MI, Astrakas L. MRI evaluation of tissue iron burden in patients with beta-thalassemia major. Pediatr Radiol 2007;37:1191–200

30. Du F, Qian ZM, Zhu L, et al. L-DOPA neurotoxicity is mediated by up-regulation of DMT1-IRE expression. PLoS One 2009;4:e593

31. Sun ER, Chen CA, Ho G, et al. Iron and the restless legs syndrome. Sleep 1998;21:371–77

32. Stocchi O, Toosy AT, Marsden JF, et al. Identifying brain regions for integrative sensorimotor processing with ankle movements. Exp Brain Res 2005 166:31–42

33. Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 1990;13:266–71

34. Khaldy H, León J, Escames G, et al. Circadian rhythms of dopamine and dihydroxyphenyl acetic acid in the mouse striatum: effects of pimocidone and of melatonin treatment. Neuroendocrinology 2002;75:201–08

35. Zywicke HA, van Gelderen P, Connor JR, et al. Microscopic R2* mapping of reduced brain iron in the Belgrade rat. Ann Neurol 2002;52:102–05

36. Petrèdes M, Pandya DN. Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and the macaque brain and corticocortical connection patterns. Eur J Neurosci 1999;11:1011–36

37. Okun MS, Fernander HH, Foote KD. Deep brain stimulation of the GPi treats restless legs syndrome associated with dystonia. Mov Disord 2005;20:500–01

38. van den Hout M, van Dijk DJ, van der Sande M, et al. Restless legs syndrome in Parkinson’s disease patients may improve with subthalamic stimulation. Mov Disord 2006;21:1287–89

39. Benabid AL, Chabardes S, Mitrofanis J, et al. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson’s disease. Lancet Neurol 2009;8:67–81

40. Gómez-Esteban JC, Tijero B, Ciordia R, et al. Factors influencing the symmetry of Parkinson’s disease symptoms. Clin Neurol Neurosurg 2010;112:302–05

41. Lu CS, Ikeda A, Terada K, et al. Electrophysiological studies of early stage corticobasal degeneration. Mov Disord 2003;18:140–46

42. Yarnitsky D, Barron SA, Benton E. Disappearance of phantom pain after focal brain infarction. Pain 1998;72:285–87

43. Saalmann YB, Kastner S. Gain control in the visual thalamus during perception and cognition. Curr Opin Neurobiol 2009;19:408–14

44. San Pedro JC, Montuza JM, Montuza JD, et al. Familial painful restless legs syndrome correlates with pain dependent variation of blood flow to the caudate, thalamus, and anterior cingulate gyrus. J Rheumatol 1998;25:2270–75

45. Hagberg N, Jääskeläinen SK, Martikainen IK, et al. Striatal dopamine D2 receptors in modulation of pain in humans: a review. Eur J Pharmacol 2004;506:187–92