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NEUROIMAGING (DJ BROOKS)

Imaging Brain Functional and Metabolic Changes in Restless Legs Syndrome

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Abstract Even though the pathophysiology of restless legs syndrome is not completely understood, several imaging studies have contributed to our understanding of the disease. Functional and metabolic impairment seems to be the pathophysiological core, tied to a single brain network or multiple connected brain networks, via neurotransmitter modifications. Positron emission tomography and single photon emission computed tomography studies support a dysfunction of dopaminergic pathways, involving not only the nigrostriatal pathway but also the mesolimbic pathway. Furthermore, a possible role of serotonergic neurotransmission has been suggested. Functional magnetic resonance imaging studies have demonstrated in restless legs syndrome patients a pathologic activation of cerebral areas belonging to both the sensorimotor and the limbic networks. Proton magnetic resonance spectroscopy has confirmed abnormality of the limbic system and suggested the presence of a glutamatergic disorder. Finally magnetic resonance studies using iron-sensitive sequences have demonstrated reduced iron content in several regions of the brain of restless legs syndrome patients. In this review we attempt to integrate all current imaging study results into a convergent pathophysiological interpretation.

Keywords Magnetic resonance imaging · Restless legs syndrome · Imaging · Functional · Metabolic · Voxel-based morphometry · Functional magnetic resonance imaging · Positron emission tomography · Single photon emission computed tomography · Magnetic resonance spectroscopy · Diffusion tensor imaging · Relaxometry · Phase imaging · Iron · Dopamine · Limbic system · Nociceptive system · Mesolimbic system · Nigrostriatal system · Sensorimotor system · Pathophysiology

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Introduction

Restless legs syndrome (RLS) is a common sensorimotor disorder, with a prevalence in the general population of 10–12% (with various studies suggesting a range of 5–20%), increasing with age and higher in women than in men [1, 2]. It is 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 [1]. Many patients also have periodic limb movements (PLMs) in sleep and wakefulness and they may complain of insomnia and/or hypersomnia [1, 2]. In 70-80% of cases it is an idiopathic disorder with no apparent cause, whereas for the remainder it has been described as a symptomatic syndrome, associated with pregnancy, uremia, iron depletion, polyneuropathy, spinal disorders, and rheumatoid arthritis [1, 3], although these conditions are probably more correctly considered “risk factors” [4]. Clinical diagnostic criteria were established by the International Restless Legs Syndrome Study Group in 1995 and were reviewed in 2003 [2, 5].

The pathophysiology of RLS is poorly understood. Clinical, neurophysiological, and pharmacological observations point towards an involvement of central nervous structures and networks, although the areas involved are somewhat uncertain, with both the dopaminergic system and iron metabolism being implicated. Several imaging studies have

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shed light on the disorder, although with some discrepancies in the findings and divergence in the interpretations offered. These studies essentially include positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies which have mainly evaluated the dopaminergic pathway, and magnetic resonance imaging (MRI) studies which have used various techniques to evaluate several putative components of the pathophysiology of RLS.

Overall, most of the imaging results point to the presence of a functional and metabolic rather than a structural impairment in the brain of RLS patients. In this article these imaging studies are summarized and their findings integrated to propose a convergent pathophysiological interpretation of RLS.

SPECT and PET Studies

SPECT and PET both use tracers labeled with radioactive isotopes to study the density of particular receptors or the regional cerebral blood flow (rCBF) and metabolism in specific areas. PET provides a higher spatial and temporal resolution than SPECT. Most nuclear medicine studies have focused on the presynaptic ([^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)tropane, [^{123}I]*N*-(3-iodopropen-2-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane, or [^{99m}Tc]TRODAT-1 in SPECT studies and [^{18}F]dopa in PET studies) or postsynaptic ([^{123}I]iodobenzamide in SPECT studies and [^{11}C]raclopride or [^{11}C]FLB 457 in PET studies) dopaminergic system in RLS patients [6–15, 16•, 17–20, 21•, 22••, 23].

SPECT Studies

The only small study that investigated rCBF in RLS patients during the state of pain induced by immobility, using technetium-99m hexamethylpropyleneamine oxime SPECT, found reduced rCBF in the caudate nuclei and increased rCBF in the thalami and anterior cingulate with increasing pain [6].

A number of studies have evaluated the binding of dopamine to its specific transporter (dopamine transporter, DAT) located in the presynaptic terminals of the neurons [7–12]. All studies but one failed to detected changes [7–11], with the single exception being the most recent study [12], which disclosed increased striatal DAT density in moderately severe old RLS patients at the level of the caudate and posterior putamen. Regarding postsynaptic dopamine (D2) receptors, three older studies [13–15] on "nocturnal myoclonus syndrome" patients, in most of them associated with RLS, detected reduced [^{123}I]iodobenzamide striatal binding as did a subsequent study on RLS patients [9]. In contrast, no change in [^{123}I]iodobenzamide binding was detected in another three studies [7, 8, 12].

Finally, a recent SPECT study used the tracer [^{123}I]2β-carbomethoxy-3β-(4-iodophenyl)tropane to study the availability of serotonin transporter in RLS patients [16•]. Although the availability of serotonin transporter in the pons and medulla was similar in the RLS and control groups, it was negatively correlated with the severity of RLS symptoms, partially suggesting a role of serotonergic neurotransmission in RLS pathophysiology [16•].

PET Studies

Only one study has used [^{18}F]fluorodeoxyglucose PET to measure cerebral metabolism in RLS patients without seeing abnormal regional metabolic uptake [17]. The presynaptic dopaminergic compartment was evaluated using [^{18}F]dopa in three studies [17–19], with no change being disclosed in one study [17] and a mild reduction of caudate and putamen uptake being disclosed in the other two [18, 19]. Recently, a PET ligand for DAT, [^{11}C]D-*threo*-methylphenidate, was used to scan patients for 90 min immediately after tracer infusion in order to evaluate membrane-bound DAT rather than total cellular DAT as in the SPECT studies (which use scans after a 24-h delay), and a decreased binding potential in putamen and caudate but not in the ventral striatum of RLS subjects was found [20].

[^{11}C]Raclopride was used mainly to study postsynaptic D2 receptors in RLS patients, and a reduced striatal uptake was demonstrated in two studies [19, 21•]. In one case, values were calculated not only for the striatal D2 receptor binding potentials per se, which were reduced, but also for the density of receptors on the membrane (β_{max}) and the receptor–ligand dissociation constant or receptor affinity (K_d), which were unchanged, together interpreted as increased level of synaptic dopamine [21•]. In a recent study, the mean magnitude of [^{11}C]raclopride binding potential was significantly lower in the mesolimbic dopamine region (nucleus accumbens and caudate) but not in the nigrostriatal dopamine region (putamen) in the RLS group, and correlated negatively with clinical severity scores and positively with the degree of improvement after dopaminergic treatment [22••]. Evidence of limbic dopaminergic impairment had also been shown by a study that used [^{11}C]raclopride to investigate the striatal region and [^{11}C]FLB 457 (a higher-affinity D2 radioligand) for the extrastriatal region, although with opposite results [23]. Indeed, this study disclosed a higher [^{11}C]raclopride binding potential at the level of the limbic and associative striatal subregions, and higher [^{11}C]FLB 457 binding potential at the level of the medial and posterior thalamus, anterior cingulate cortex, and insulae in RLS patients compared with controls [23]. All these brain structures serve the medial nociceptive system, which is

thought to regulate the affective-motivational component of pain. The involvement of limbic structures in RLS is also supported by the finding that in orbitofrontal and anterior cingulate cortex, the binding levels of the nonselective opioid receptor radioligand [^{11}C]diprenorphine were negatively correlated with RLS severity [24].

MRI Studies

Functional MRI

Functional MRI (fMRI) is an imaging modality based on the blood-oxygen-level-dependent contrast, using hemoglobin as a naturally occurring endogenous contrast agent. It is used to measure brain activity following an adequate stimulus or at rest by detecting associated changes in blood flow, based on the observation that cerebral blood flow and neuronal activation are coupled [25].

The first fMRI study in RLS patients reported activation of specific brain areas associated with the occurrence of sensory and/or motor symptoms of RLS [25]. The patients were investigated under four different conditions: (1) during a symptom-free period; (2) during sensory symptoms of the legs; (3) during the occurrence of PLMs and sensory symptoms; (4) while mimicking PLMs. An activation of the thalamus was demonstrated during leg discomfort of red nuclei and brainstem during PLMs, and of cerebellum during both conditions [25]. A subsequent fMRI study used simultaneous recording of EMG activity and fMRI in seven RLS patients instructed not to move voluntarily [26]. First, a negative correlation of tonic EMG and the degree of sensory leg discomfort was found. Second, the tonic EMG was associated with activation in areas associated with motor and somatosensory pathways, including precentral and postcentral gyri and cerebellum, but was also correlated positively with activation of the posterior cingulate gyrus and negatively with activation of the anterior cingulate gyrus [26]. Unfortunately, healthy subjects were not included in this study, limiting the conclusions about the significance of these results for RLS pathophysiology [26]. Another fMRI study, using a motor paradigm consisting in alternating active plantar flexion and dorsiflexion, found activation in primary motor cortex, primary somatosensory cortex, somatosensory association cortex, and the middle cerebellar peduncles in both RLS patients and controls, and in the thalamus, putamen, middle frontal gyrus, and anterior cingulate gyrus of the RLS patients only [27].

The same group performed a more recent fMRI study on RLS patients during nighttime episodes of sensory legs discomfort and PLMs [28•]. The patients were observed to activate the primary motor and somatosensory cortex, the thalamus, pars opercularis, and ventral anterior cingulum in the left hemisphere, the striatum, inferior and superior parietal lobules, and dorsolateral prefrontal cortex in the right hemisphere, and the cerebellum, midbrain, and pons bilaterally [28•]. It was suggested that activation of the striatofrontolimbic area may represent the neurofunctional substrate mediating the repetitive compulsive movements seen in RLS [28•].

Structural and Microstructural Studies

Conventional brain MRI does not disclose any structural abnormalities in idiopathic RLS patients. Magnetic resonance studies using advanced techniques such as voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) have searched for subtle structural changes but have reported contrasting results [28•, 29–36]. VBM is very sensitive in detecting brain structural changes by measuring tissue volumes on high resolution 3D T1 weighted images, and voxelwise analysis of diffusion tensor images can disclose microstructural alterations by calculating mean diffusivity, axial diffusivity, radial diffusivity, and fractional anisotropy, parameters all sensitive to neuronal and/or glial integrity [35]. The first VBM study of RLS patients detected bilateral gray matter increases in the pulvinar that were interpreted as a consequence of a chronic increase in afferent input of behaviorally relevant information [29]. Later VBM studies did not confirm this result. One study found significant regional decreases of gray matter volume in the bihemispheric primary somatosensory cortex and left-sided primary motor areas [30], another found a slight increase in gray matter density in the ventral hippocampus and middle orbitofrontal gyrus [31], and another found reductions in white matter volumes in small areas of the genu of the corpus callosum, anterior cingulum, and precentral gyrus [32], while four other studies yielded no specific gray matter alterations [28•, 33–35].

One DTI study of RLS disclosed multiple bilateral subcortical areas of significantly reduced fractional anisotropy, mainly close to the primary and associated motor and somatosensory cortices [36]. This study was performed in a subgroup of the RLS patients recruited for the VBM study of Unrath et al. [30], who suggested that both works gave support to an altered sensorimotor network [36]. These findings were not replicated by a subsequent DTI study which disclosed no change [35].

Methodological differences in the analysis, heterogeneity in terms of size and clinical features of the samples studied, and the possible detection of secondary alterations contribute to the discrepant results obtained in the various studies.

^{1}H Magnetic Resonance Spectroscopy Studies

Magnetic resonance spectroscopy (MRS) is a noninvasive method that permits measurement of the concentration of

specific biochemical compounds in the brain in precisely defined regions guided by MRI [37••, 38•]. With MRS, spectra of many biologically important metabolites can be quantified. ^{1}H-MRS is the most used in clinical practice . ^{1}H-MRS can detect *N*-acetylaspartate (NAA)-containing compounds, choline-containing compounds, creatine–phosphocreatine (Cr), glutamate and glutamine (Glx), *myo*-inositol, *scyllo*-inositol, and lipids. The quantity of metabolite is usually reported relative to that of total creatine, which is assumed to be relatively constant [37••, 38•].

Two studies have used ^{1}H-MRS in RLS patients, both examining the thalamus although localizing different subregions of this structure and with different primary hypotheses [37••, 38•]. The aim of the first study was to evaluate the metabolism of the medial thalamus, as part of the limbic and nociceptive system, in idiopathic RLS patients [37••]. After structural thalamic changes had been excluded using a multimodal magnetic resonance approach, including DTI, VBM, and volumetric and shape analysis focused on the thalami, ^{1}H-MRS revealed a medial thalamic NAA/Cr reduction in the patients interpreted as a metabolic impairment rather than a neuronal loss and highlighting the possible role of limbic system dysfunction in the pathophysiology of RLS [37••].

The second study investigated the whole right thalamus of RLS patients, disclosing an increase of the Glx/Cr ratio in patients compared with controls [38•]. The Glx/Cr ratio correlated with the wake time during the sleep period and all other RLS-related polysomnographic sleep variables except for the number of PLMs per hour [38•]. The authors of the study suggested the presence of a glutamatergic disorder in RLS which could underlie the arousal sleep disturbance and not the PLMs, which would be more strongly related to the dopaminergic system, in a hypothesis of dual mechanisms potentially underlying the clinical abnormalities seen in RLS [38•].

Iron-Sensitive Sequences

It has been well documented through in vitro studies that paramagnetic iron will proportionally increase proton transverse relaxation rates. Furthermore, ferritin and hemosiderin are considered to be the only forms of non-heme iron present in sufficient quantities to affect magnetic resonance contrast in the human brain [39]. The most iron sensitive parameters are T_2* or T_2', and T_2 to a lesser extent. Relaxometry is frequently used to evaluate the different relaxation rates R_2 ($1/T_2$), R_2* ($1/T_2$*), and R_2' ($1/T_2' = R_2* - R_2$) [39]. Some magnetic resonance studies [27, 28•, 40–42] have used these sequences to quantify the iron level in RLS patients, and almost all results [27, 40–42] were in line with the hypothesis of reduced iron content in several regions of the brain of RLS patients, although with some discrepancies. In two studies by the same group, regional brain iron concentration was assessed in RLS patients by R_2' measurement, and the mean iron content of the substantia nigra was significantly lower only in the early-onset RLS patients (younger than 45 years) [40, 41]. In a study performed in patients with late-onset RLS, the T_2 relaxation time was assessed separately for the two components of the substantia nigra, and low iron content was found in the pars compacta, but not in the pars reticulata [27]. The same authors subsequently showed an unexpectedly decreased T_2 relaxation time in the right internal globus pallidus and the subthalamic nucleus of untreated patients with early-onset RLS, indicating increased iron content, without any change in the substantia nigra [28•]. Another group, without differentiating between early-onset and late-onset cases, found that the mean T_2 values of multiple regions were higher in RLS patients, although they were significantly increased in four regions (caudate head and medial, dorsal, and ventral thalamus) [42].

Recently a new technique, phase imaging, has been developed that uses differences in magnetic susceptibility to generate a tissue-unique contrast, which correlates directly with iron content [39]. The findings of a phase imaging study in RLS patients support reduced iron content in the substantia nigra, thalamus, putamen, and pallidum [43].

Using a different technique, transcranial B-mode sonography, some authors have reported hypoechogenicity of the substantia nigra [42, 44–47], red nucleus, and brainstem raphe [46] of RLS patients. Substantia nigra hypoechogenicity inversely correlated with T_2 values [42] and was interpreted as secondary to iron deficiency.

Globally, these results are consistent with a diffuse but regionally variable low brain iron content in idiopathic RLS patients, notwithstanding some discordances which could be explained by clinical inhomogeneity or by technical considerations as T_2, unlike T_2' or phase contrast, also depends on tissue water content and other local microstructural changes that can affect relaxation times [39].

Conclusion

A number of imaging studies have been performed in RLS patients in recent years in an attempt to improve understanding of the basis of the disease. Somewhat inhomogeneous findings are justified by possible clinical differences in the samples studied and in the broadly variable technical parameters used in the different methodological approaches applied.

First, to date no clear structural or microstructural abnormality has been demonstrated in the brain of RLS patients [28•, 29–36]. The variable abnormalities reported in a few works may be due to random or secondary brain modifications [29–32, 36]. Accordingly, a functional and metabolic impairment seems to be the pathophysiological core of the disease. This impairment seems to be tied not to a single brain region

but rather to a network or even several connected networks, via a single or probably multiple neurotransmitter modifications.

PET and SPECT studies consistently support a dysfunction of dopaminergic pathways, involving not only the nigrostriatal pathway but also the mesolimbic pathway [9, 12–15, 18–20, 21•, 22••, 23]. The type of dysfunction has not been fully clarified. Findings of hyperfunction may be explained by an increase in the level of synaptic dopamine [9, 12–15, 19, 21•, 22••], although a more complex change is possible, with some regions presenting hyperdopaminergic and others presenting hypodopaminergic activity.

Functional MRI (fMRI) studies have demonstrated an activation of cerebral areas belonging to the sensorimotor network [25–27, 28•] and also to the limbic network [26, 27, 28•]. An involvement of the limbic structures in RLS patients is also supported by metabolic alteration in the medial thalami disclosed by H^1-MRS [37••], by technetium-99m hexamethylpropyleneamine oxime SPECT [6], and by the limbic dopaminergic receptor alteration detected by PET studies [22••, 23]. From a behavioral point of view, considering the compulsive urge to move of RLS patients, the limbic system may be implicated in the pathophysiology of the disorder. Equally, the overlap of the limbic system with the nociceptive system and its role in the affective-motivational sensorimotor processing of painful sensory inputs [48] are suggestive of a dysfunction of the nociceptive system. In this regard, sensory descriptors in RLS can be similar to those of neuropathic pain [49], and the opioid receptor level in the medial nociceptive system correlated negatively with RLS severity [24]. Furthermore, limbic dysfunction could explain the greater propensity of RLS patients to develop depressive and anxiety disorders [50–53], mild cognitive deficits [53–56], and sympathetic overactivity [57].

Another consistent finding is the lower brain iron content in RLS patients, as supported by most of the MRI studies using iron-sensitive sequences [27, 40–43]. This is in agreement with the hypothesis linking iron and dopamine [58], a concept supported by pathological data and animal models [59].

Although some of the above-mentioned observations should be confirmed in future studies in order to exclude chance or secondary findings, a convergent interpretation of all current results can be tentatively suggested (Fig. 1). The *primum movens* may be a primary reduction of brain iron content in RLS patients leading to a dysfunction of mesolimbic and nigrostriatal dopaminergic pathways, and possibly of other neurotransmitter systems. Indeed, animal models [59–61] have demonstrated that iron deficiency affects not only the dopamine but also the serotonin and glutamate levels, which two imaging studies have already linked to RLS [16•, 38•], and noradrenalin levels, as yet unstudied by imaging techniques. The dysfunction of the dopaminergic system or otherwise is reflected in a dysregulation of the limbic network and simultaneously and/or subsequently at the level of the sensorimotor network, finally resulting in the abnormal sensorimotor and other manifestations.

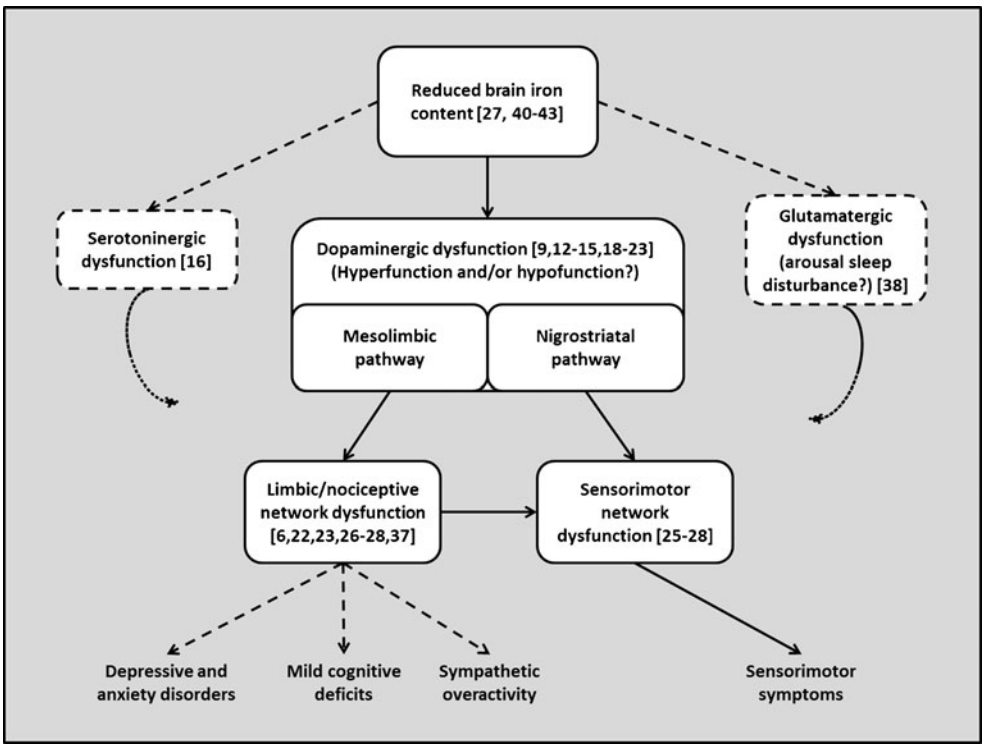

Fig. 1 Proposed schematic integration of currently available imaging findings in restless legs syndrome patients into a unitary pathophysiological hypothesis. *Dotted lines* are used for the less studied research fields. References to studies for each step are indicated in *brackets*. See the text for details

Compliance with Ethics Guidelines

Conflict of Interest Giovanni Rizzo has received grant support from Neureca Onlus.

Caterina Tonon, David Manners, Claudia Testa, and Raffaele Lodi declare that they have no conflict of interest.

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