Abstract

**Background and purpose:** The evaluation of the pathophysiology of restless legs syndrome (RLS) stems largely from recognition of the information provided by both pharmacological treatment of the disorder and the secondary forms of the disorder. This article examines the pathophysiological implications of each of these clinical aspects of RLS.

**Patients and methods:** The article reviews the existing literature in relation to possible pathology suggested by the clinical data. It will then explore other data supporting each of the possible pathologies and examine the relationships between these pathologies.

**Results:** The pharmacological treatment data strongly support a dopaminergic abnormality for RLS. Other pharmacological data and some imaging data also support this, although the data are not entirely consistent. The secondary forms of RLS strongly support an iron deficiency abnormality for RLS, further documented by several other studies. Some animal studies have shown a relation between iron deficiency and dopaminergic abnormalities that have some similarity to those seen in the RLS patient.

**Conclusions:** It is concluded that there may be an iron–dopamine connection central to the pathophysiology of RLS for at least some if not most patients with this disorder.

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**Keywords:** Restless legs syndrome; Dopamine; Iron; Pathophysiology; Ferritin; CSF

1. Introduction—development of the RLS program at Johns Hopkins University

The involvement of the Johns Hopkins group in the study of the restless less legs syndrome (RLS) started with a review of our clinical sleep medicine cases that I conducted in 1988–1989. When I reviewed the cases I found a large number that had been diagnosed with periodic limb movement disorder (PLMD) who had in general not benefited from our treatment, which was entirely restricted to the use of benzodiazepines and some behavioral sleep treatments. Some complained of having an expensive test and no help. More than half had discontinued their medications. In the face of these discouraging results I did a review of the literature regarding periodic limb movements in sleep (PLMS) and PLMD and realized we had failed in our sleep medicine program to appreciate that the RLS could be a major cause of PLMS. We were all aware that this connection had been made many years earlier by Lugaresi et al. [1], but had simply considered the PLMD diagnosis to be not only more common but also clinically more significant. At that time, however, there was no well-established diagnostic criterion for RLS and no awareness of its prevalence or clinical significance. The sleep education provided by the professional organization now recognized as the American Academy of Sleep Medicine provided little information or education about RLS. The limited education provided about RLS (e.g. two slides in a 1-h talk) recommended treatment based on current literature with opiates [2], clonazepam [3] or carbamazepine [4–7], specifically recommending avoidance of dopaminergic agents because they were expected to exacerbate the condition, based on reports of effects on myoclonus [8]. Professional and scientific interest focused on the periodic limb movements of sleep (PLMS). RLS was a casual afterthought and the impression given was that it was difficult to treat and not a common problem.
The recent literature at the time of our review also revealed that Akpinar [9,10], who was unaware of the recommendation to avoid the use of levodopa to treat RLS, had described a serendipitous finding to the contrary. He reported that levodopa provided dramatic and almost complete relief of RLS symptoms for his patients, maintained with continued use of the medication. The efficacy of this treatment had been further documented by the excellent studies of Montplaisir and colleagues [11,12]. At the same time, however, Walters, Hening and Kavey were reporting effective treatment of RLS with opioids including propoxyphene [13]. My first goal, then, was to determine which of these treatments we should use and whether they were suitable for all patients with PLMD or only those with RLS. We conducted a double-blinded crossover pilot study to determine the relative efficacy of carbidopa/levodopa and propoxyphene for treatment of patients with PLMS, provided they had RLS or significant daytime complaints indicating that PLMS disturbed their normal functioning. We excluded patients with other sleep disorders. Almost all selected patients had RLS, and to our surprise all showed dramatic reduction in PLMS on a very low dose of either 50/100 or 100/200 carbidopa/levodopa and only marginally significant reductions on either 100 or 200 mg of propoxyphene [14,15]. We concluded that dopaminergic medications should be considered as the treatment of first choice for RLS, and possibly for PLMD contingent on the patient showing significant daytime dysfunction associated with the PLMS. Moreover, we agreed with Montplaisir that the excellent pharmacological response at a very low dose suggested specific dopaminergic pathology for RLS. This appeared to be a potentially significant area for further study. At that time we decided to focus attention on RLS and its pathology, and this effort was greatly enhanced when Dr Christopher J. Earley joined the clinical and research team and committed to advancing its work.

Further examination of all the data on RLS provided two different bases for assessing potential pathophysiology: pharmacological response and secondary forms of RLS. The first strongly supports the dopaminergic abnormality, and this has been the emphasis of most studies to date. The second, however, fails to provide any support for RLS as a primarily dopaminergic disorder, but rather posits the clearly established secondary causes of RLS as iron deficiency anemia, end stage renal disease, and pregnancy, all involving iron insufficiency. (Note that although diabetes and neuropathy are commonly given as causes for secondary RLS, the data supporting this claim do not currently exist.) There are also many other reported causes of secondary RLS, but none aside from these three have been clearly documented, with the possible exception of gastric surgery. The following provides a review of the evidence, separately for each of the suggested pathophysiologies for RLS, and then briefly examines some possible connections between these two pathologies noting some of the predictions and research gains from a putative dopamine–iron connection in RLS.

2. Evidence for dopaminergic pathology in RLS

The strongest evidence for a dopaminergic pathology in RLS comes from the pharmacological response to medications. To date, all evaluated agents that enhance dopamine activity have been found to reduce RLS symptoms in open-label uncontrolled trials. Double-blinded, placebo-controlled studies have demonstrated the treatment benefit for levodopa [12,16,17] and for the currently used major dopamine agonists, i.e. pergolide [18,19], pramipexole [20] and ropinirole [21]. These medications are effective at remarkably low doses relative to that given for Parkinson’s disease, sometimes at the minimum tablet size available to the patient, e.g. carbidopa/levodopa at 25/100 or pergolide at 12.5 mg. Moreover, they are effective immediately after the first therapeutic dose for virtually all patients with RLS. The immediate, universal and almost complete reduction of RLS symptoms by low doses of levodopa provides strong support for a possible dopaminergic abnormality in this disorder.

Dopamine antagonists generally exacerbate RLS symptoms, suggesting a primary role for the dopamine system. In the first report of this effect, Pimozide, a dopamine antagonist was shown to reverse treatment benefits of the opioid Codeine in one patient [22]. In a later study, Metoclopramide given IV produced increased RLS symptoms on a suggested immobilization test in five out of six subjects evaluated [23] and produced an increase in prolactin release [23] that was at least three times greater than that expected from age-matched normals [24]. Dopaminergic abnormalities in the tuberoinfundibular system have been further supported by the exaggerated response of prolactin to levodopa [25].

It deserves note that all other types of medications used to treat RLS require doses equivalent to those used to treat other conditions. The doses for the opioids are usually in the same range as that used for the analgesic treatments; e.g. the average effective dose of oxycodone in one double-blind controlled study was 15.7 mg (about three 5 mg tablets) [26]. Similarly, the average effective dose of the anticonvulsant gabapentin in a double-blind controlled study was 1,855 mg [27], about the same as that used for seizure control. The effects of these medications on RLS symptoms may therefore be indirect, such as a non-specific effect on nociception. Moreover, naloxone does not exacerbate RLS symptoms, and when it was given IV did not produce increased symptoms during a SIT test [23]. Thus, the pharmacological evidence supports a dopaminergic abnormality more than it does for any other neurotransmitter system.

Imaging studies have produced mixed results. The one PET study with an adequate sample size found decreased...
striatal D2R binding [28]. Two of four SPECT studies also found decreased striatal D2R binding [29,30], but one of these studies [30] was compromised by having significantly younger controls than patients; thus the well established decrease in D2R binding with age could explain these results. The other study had no similar problem and was particularly well controlled. Two other similarly well-controlled SPECT studies, however, failed to find any difference in striatal D2R binding [31,32]. Two SPECT studies failed to find differences in striatal dopamine transporter (DAT) [29,32]. But it should be noted that all of these studies were conducted in the daytime when RLS symptoms are minimal. It may be that evaluation of the dopaminergic system when the patient has few symptoms will produce variable results depending on the degree of symptom expression, thus explaining the conflicting results reported above. Evaluation during the symptomatic period is needed, but has not yet been done. There is one report of a PET study showing significant circadian changes in D1 receptor binding [33], and it seems reasonable to assume the same may occur for D2 receptors. Moreover, DAT plays a significant role in the substantia nigra in regulating the nigrostriatal dopaminergic system, and this has not yet been studied. There have also been two good studies using F-dopa PET that both found significantly less uptake by about 11% in the putamen for RLS patients compared to normal controls [28,34]. One of these studies reported approximately the same difference (P < 0.05) for the caudate [34], while the other found no significant difference for the caudate [28]. These studies are somewhat difficult to interpret. They suggest decreased pre-synaptic uptake, but it deserves note that in Parkinson’s disease even patients with significant cell loss have normal F-dopa striatal uptake. This small decrease may reflect changes in the status of the growth or integrity of the terminals of the dopaminergic cells. Since fluorodopa is also taken up by serotonergic cells there may be some uncertainty regarding the specificity of this finding for the dopaminergic cells. Finally, there is a problem with competitive binding for receptor uptake. An increase in extracellular dopamine would produce a decrease in dopamine receptor binding. The currently available data do not permit controlling for this possibility. Overall, the imaging data, while not entirely consistent, available data do not permit controlling for this possibility. They suggest decreased pre-synaptic uptake, but it deserves note that in Parkinson’s disease even patients with significant cell loss have normal F-dopa striatal uptake. This small decrease may reflect changes in the status of the growth or integrity of the terminals of the dopaminergic cells. Since fluorodopa is also taken up by serotonergic cells there may be some uncertainty regarding the specificity of this finding for the dopaminergic cells. Finally, there is a problem with competitive binding for receptor uptake. An increase in extracellular dopamine would produce a decrease in dopamine receptor binding. The currently available data do not permit controlling for this possibility. Overall, the imaging data, while not entirely consistent, support some mild reductions in the nigrostriatal dopaminergic system, but further study is needed to confirm this and better describe the characteristics of this abnormality.

3. Evidence for iron pathology in RLS

The potential central role of iron insufficiency to RLS is indicated primarily by the secondary causes of RLS, but also from some limited pharmacological study. Three causes of secondary RLS have been well established. RLS develops with the development of each of these disorders, is more common for patients with these disorders than expected for the population, and disappears when the disorders are treated. End stage renal disease (ESRD) with dialysis is perhaps the best studied of the secondary causes of RLS. RLS develops with the disorder and occurs in 20–60% of patients on dialysis [35–37], or about 2–6 times more frequently than expected. The RLS symptoms with ESRD have been related to increased mortality [35]. Kidney transplants resolving the renal disease also lead to a disappearance of all RLS symptoms within 1–21 days after transplant for all patients who have been reported [38,39]. RLS also develops in about 20–30% of pregnant women [40,41]. Women with RLS before pregnancy reported increased severity of RLS symptoms during pregnancy [41]. The RLS symptoms are clinically reported to usually resolve after pregnancy, and did so in 85% of the cases in one study [40]. Ekbom [42] identified RLS as commonly occurring with iron deficiency anemia and this has gained wide clinical acceptance, although there have been few studies documenting this relationship. O’keeffe found that oral iron supplement treatment of iron deficiency among older patients with RLS reduced or reversed the RLS symptoms [43]. Data indicate that two other conditions increasing the risk of RLS are gastric surgery and frequent blood donations. The increased prevalence of RLS after gastrectomy was described by Ekbom [44] and supported by subsequent study [45]. More recent studies have documented the increased prevalence of RLS for frequent blood donors [46]. Both neuropathy and diabetes have been reported to be associated with the development of RLS, but survey studies using full diagnostic criteria have failed to find any significant increase in the prevalence of RLS for either neuropathy [47] or diabetes [48].

All of the established causes of secondary RLS involve compromise of iron sufficiency. Treatment with IV iron and erythropoietin reduced the RLS symptoms of PLMS in one study of patients on dialysis [49]. Iron deficiency commonly occurs in pregnancy, and the pregnant women at risk of RLS showed compromise in iron status before pregnancy [40], although it was noted that folate may also be involved. Correcting iron status in patients with iron deficiency has been found to reduce RLS symptoms [43]. Both gastric surgery and frequent blood donations have a well-recognized increased risk of reduced iron sufficiency. Virtually all the conditions clearly associated with common occurrence of secondary RLS involve problems with iron metabolism, and the opposite is also true; all conditions involving iron insufficiency increase the risk of developing RLS.

The relationship between iron and RLS was recognized much earlier by Nordlander, who postulated that a tissue deficiency in iron might be responsible for the occurrence of RLS. He also used IV iron to treat RLS patients, including several without clear anemia, and reported complete remission of symptoms lasting several months for 21 of the 22 patients. [50,51]. Thus, pharmacological data as well
as data from secondary RLS strongly support a relation between iron deficiency and RLS.

4. Evaluation of iron status in RLS

If we assume that the primary pathology of RLS involves iron insufficiency, then we want to measure the iron status and relate it to the RLS status. Our group at Johns Hopkins and Hershey School of Medicine has explored four different methods for evaluating the iron status of RLS patients; i.e. serum measures of ferritin, CSF measures of ferritin and transferrin, magnetic resonance imaging (MRI) measures of regional brain iron concentrations and autopsy evaluations of brain iron status. All of these have supported the view that there is a brain iron deficiency in RLS patients.

The serum ferritin had previously been found by O’Keeffe to correlate with RLS symptoms for older patients. O’Keeffe correctly recognized that serum ferritin provides the best measure of peripheral iron stores. Serum measures of iron and transferrin relate more to immediate activity of iron transport and only very poorly to the status of the body stores of iron. Even the serum measure of the soluable transferrin receptor [52], when in the normal range, relates to the rate of erythropoesis more than to iron stores, although when abnormal provides a more reliable measure of the iron deficiency. Ferritin, in contrast, relates to iron stores over a wider range with the exception of the elevated values seen in response to disease states. We decided to replicate the O’Keeffe finding and extend it to a full age range of RLS patients. For our study we also developed and validated a fairly simply clinical rating scale for RLS severity, the Johns Hopkins Restless Legs Scale (JHRLSS). This scale assessed RLS severity by the patients’ report of the usual time of symptom onset, with 0 indicating no symptoms, 1 symptoms only at or after bedtime, 2 symptoms in the evening (after 6 p.m.) but not earlier, and 3 symptoms earlier in the day. This scale correlated significantly with the objective polysomnographic measures of sleep disruption from RLS of PLMS/h of sleep and sleep efficiency [53] and has been shown to be responsive enough to detect treatment benefits in small-size, double-blinded medication trials [54]. In a consecutive series of available RLS patients we found approximately the same degree of correlation between our subjective scale for RLS severity and serum ferritin (r = 0.48, P = 0.02; see Fig. 1) [55] as was reported by O’Keeffe for his older RLS patients.

In a subsequent study, morning CSF iron measures were obtained from 16 consenting RLS patients and eight age-matched normal controls. Studies with iron-deprived rats indicate that low CSF ferritin and high transferrin can be expected to occur with reduced brain iron. The RLS patients compared to controls showed differences for both iron status measures in the CSF (P < 0.02), despite having normal serum levels for all iron measures, and all 16 patients were abnormal in at least one of these CSF measures. Thus, all RLS patients in this study showed abnormally reduced CNS iron status. A particularly striking finding from that study was the positive correlation between serum and CSF ferritin. Higher levels of ferritin in the serum were associated with higher levels in the CSF for both normal controls and RLS patients, but the slope of this relationship was much lower for the RLS patients. Thus, for RLS patients, higher levels of 200 mcg/l or greater for serum ferritin tended to occur with CSF ferritin in the low range of the usual normal levels.

The MRI has long been known to be altered by the amount of iron in ferritin, and recent studies have developed a valid MRI measure of regional brain iron concentration. For this measure the transverse relaxation rates or the inverses of the relaxation times are used, and it has been found that $R_2$, representing the relaxation resulting from field inhomogeneities that can be reversed by an 180° pulse, provides the most specific measure of regional brain iron. $R_2$ is measured as the irreversible transverse relaxation rate and $R_2^*$ represents the sum of $R_2$ and $R_1$. The images with $R_2^*$ will have the most sensitive measure of iron content and are used for identification of significant regions, but the values of $R_2^*$ are the most specific for iron since they will not be contaminated by other factors affecting $R_2$ relaxation rates such as brain water content [56]. In our study of five RLS patients and five age-matched controls the RLS patients showed significant decreases in $R_2$ in the substantia nigra, which correlated significantly with the JHRLSS measure of severity [57]. The much lower iron content in the substantia nigra can be clearly seen for the RLS patient compared to the control (shown in Fig. 2). Thus, the brain iron problems for RLS involve particularly significant iron reduction in the substantia nigra, which appears not only to be related to occurrence of RLS but also to its severity.

Finally, a recent initial report of an autopsy analyses of the substantia nigra for an RLS patient compared to controls showed reduced iron content in the cells, an apparent
reduction in H-ferritin along with an abnormal cellular distribution of L-ferritin [58].
The combined impact of all these studies can leave little doubt that at least some, and probably most RLS patients have an abnormality of iron metabolism that appears central to the pathology of the disorder. The question remains about the connection between these two pathologies for RLS: iron and dopamine.

5. Possible connections between iron and dopamine pathology in RLS

Iron is known to be a co-factor at the rate-limiting step in the production of dopamine, and decreased iron may reduce the tyrosine hydroxylase activity. This, however, was not clearly shown in the autopsy study [58], and the role of iron in this step may not be very sensitive to iron loss. Iron deprived rats with brain iron deficiency have been found to have decreased D1 and D2 receptors [59], decreased DAT [60], and increased extra-cellular dopamine in the striatum [61] and a more consistent decrease in H- than L-ferritin in brain regions [62]. As noted above, two imaging studies of RLS patients suggest a possible decrease in the D2R binding [28,29], which could reflect a decrease in the D2 receptors, an increase in extra-cellular dopamine, or both. The autopsy studies have also shown a greater decrease in H- than L-ferritin [58]. These results are in general consistent for both the RLS patients and the effects of iron deprivation on dopaminergic function in the rat brain. The findings for the DAT are, however, not the same for the rat iron-deficiency and human RLS, but the DAT studies for RLS patients involved only daytime evaluations and did not use the more sensitive PET measures. These need to be extended before it is clear that this is a real difference. The similarities of the dopaminergic abnormalities resulting from brain iron deprivation in the rat and those observed to occur in RLS patients indicate a possible strong iron-dopamine connection in RLS pathology that should be further explored.

It is particularly interesting to note that most of these animal studies have neglected to examine the circadian effects. One study evaluated changes in the extra-cellular dopamine of normal and iron-deprived rats over the time period from the last part of the rest period through the first part of the active period (from light to dark periods). The normal animal showed a significant increase in extra-cellular dopamine, but the iron-deprived rat showed an increase that was four times greater [61]. This is particularly hard to explain given that motor activity is, if anything generally decreased for these iron-deprived animals during the active period. The issue of circadian variation in dopamine function in relation to iron deficiency deserves to be more carefully explored, especially given the pronounced circadian pattern of RLS symptoms.

6. Conclusions

The data reviewed above provides support for the view that iron and dopamine may be linked in producing RLS symptoms. Further evaluation of the iron–dopamine connection in general, and particularly in relation to RLS, is needed.

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References


