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The Role of Iron in Restless Legs Syndrome

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Abstract: The impressive relief from restless legs syndrome (RLS) symptoms provided by levodopa treatment indicates RLS is caused by a dopaminergic abnormality. But similar and more lasting relief also occurs for iron treatment in some patients. Thus there are two major putative causes for RLS: CNS dopaminergic abnormality and CNS iron insufficiency. This article presents the data documenting that both peripheral and CNS iron insufficiency occur with RLS symptoms. Brain iron insufficiency is supported by independently replicated cerebrospinal fluid and brain imaging studies for patients without iron deficiency (ID) anemia. Autopsy studies and intravenous iron treatment further link brain iron insufficiency to RLS. The brain iron insufficiency in patients with RLS is now well established. In this article the data are reviewed that support the following postulates combining dopaminergic and iron causes of RLS: (1) All conditions that compromise iron availability will increase the risk of RLS leading to a higher than expected prevalence of RLS in these conditions. (2) Some patients with RLS have marginal CNS iron status that can become insufficient when deprived of normal access to adequate peripheral iron or may be insufficient even with normal access to adequate peripheral iron. (3) The change or reduced CNS iron status produces RLS symptoms largely through its effects on the dopaminergic system and the corollary to 3. (4) Dopaminergic system abnormalities producing RLS symptoms will be included in those produced by brain ID. Study of the iron model of RLS offers hope for developing new treatment approaches and perhaps methods to prevent or cure the disorder. © 2007 Movement Disorder Society

Key words: iron deificieny; restless legs; dopaminel intravenous iron.

When Ekbom provided the first modern medical descriptions of restless legs syndrome (RLS), he noted a high prevalence of iron deficiency (ID) among patients with RLS.[1] This striking relation led a contemporary of Ekbom, another Swedish neurologist Nordlander, to not only propose that ID in some body tissue caused RLS but also to successfully treat 21 0f 22 patients with RLS with relatively large doses of intravenous (IV) iron.[2,3] The modern discovery of the remarkably effective dopaminergic treatment for RLS has led to an emphasis upon finding a dopamine pathology in RLS using techniques similar to those for the study of Parkinson's Disease. These studies have generally failed to find convincing evidence in replicated studies for dopamine pathology in RLS. That direction in research largely ignored considerations of the relation of iron to RLS despite the clinical evidence showing that ID produces RLS; all conditions that compromise iron status increase the risk of RLS and iron treatments reduce or even cure RLS. Moreover, autopsy, cerebrospinal fluid (CSF), and brain imaging studies document low CNS iron status for patients with RLS. In this chapter, we review the iron and RLS connections as found in clinical, peripheral, and CNS studies and then advance the iron model of RLS and discuss its implications for unraveling the neurobiology of RLS.

CLINICAL INDICATIONS OF THE RLS–IRON CONNECTION

There are three major secondary causes of RLS: ID, end-stage renal disease, and pregnancy. In each of these there is a higher than expected prevalence of RLS, but even more important is that RLS starts after these conditions start and commonly resolves when the condition is corrected. These very disparate conditions share at least one common problem. They all compromise iron sufficiency and in each case when the condition is cor-

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rected the iron problem is also corrected. The iron insufficiency and not other problems in these disorders appear to produce the RLS. Thus, end stage renal disease produces neuropathy but that neuropathy changes little with successful kidney transplant, yet the RLS usually completely disappears.[4,5] Moreover, high doses of IV iron reduce the RLS symptoms in patients with end stage renal disease.[6] Similarly, the decreased blood volume that occurs with delivery provides a rapid improvement in access to iron stores and there is a corresponding rapid remission of any RLS symptoms.[7] Increasing the body iron stores in patients with RLS and ID can provide complete relief from all RLS symptoms in some patients.[8] These considerations led to our hypothesis: *All conditions that compromise iron availability will increase the risk of RLS leading to a higher than expected prevalence of RLS in these conditions.*

This hypothesis has been proven correct for all such conditions evaluated to date, eg: Gastric surgery[9] and low-density lipoprotein apheresis.[10,11]

It deserves note that the serology measures of iron status, thought the most frequently used, have limitations. They neither provide a direct measure of cellular or bone marrow iron status nor an accurate measure of neuronal iron status. Despite these problems, one community based study found higher serum transferrin receptor indicating iron compromise for RLS compared to control subjects[12] but this was not found in another similar study.[13]

IRON STATUS AND RLS

Peripheral Iron Status and RLS

Serum ferritin provides the generally accepted best single measure of iron stores.[14,15] The usually published normal values for ferritin, however, tend to confuse iron status evaluations since they represent population samples not biological norms. When serum ferritin values were compared to iron status from the bone marrow in patients thought to be iron deficient the response–observer curve indicated an optimum cut-off of about 45 μg/L with ferritin below that indicating low peripheral iron stores (see Fig. 1).[15] The phase-reactive property of ferritin, however, sometimes produces falsely elevated values even in the face of ID. Percent transferrin saturation below 20% or TIBC above 400 μg/dL both also indicate ID and should be used clinically given the risk of falsely elevated ferritin values. Serum transferrin receptor provides a useful alternative to ferritin for younger subjects. It does not have the phase reactive aspect of ferritin and thus is a more sensitive measure, but unfortunately a less specific indicator of low iron

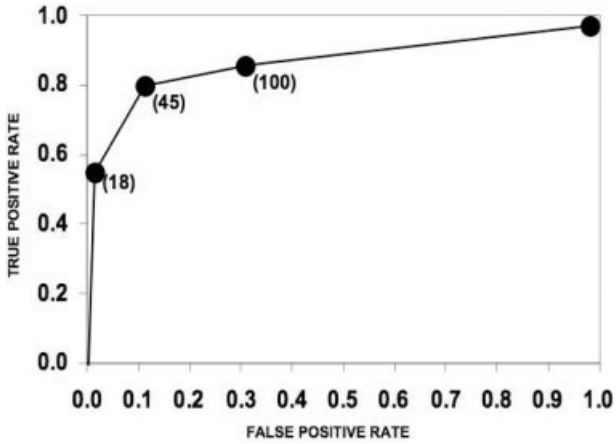

FIG. 1. ROC curve for serum ferritin criteria values (indicated in parentheses) for those below that value considered to be anemic giving for each value the percentage with anemia correctly identified and the percentage of those identified with anemia who do not have it. Anemia was determined by bone marrow aspiration from 259 consecutive eligible and consenting patients with suspected anemia. (Reproduced with permission from Guyatt GH, et al., Am J Med, 1990, 88, 205–209, © Excerpta Medica).

than ferritin.[16] TfR also fails to discriminate between ID and anemia of chronic disease, and therefore is not a useful test for the elderly.[17] Given the ferritin measurement problems it has been recommended that patients with RLS with ferritin of 50 μg/L or less be considered for oral iron treatment.[18]

Serum ferritin somewhat indirectly reflects body iron stores and in two studies correlated with RLS severity.[19,20] Moreover, patients with RLS who develop ID show marked exacerbation of their symptoms. Thus, any sudden worsening of RLS symptoms unrelated to medication changes has to be considered as likely indicating blood loss or other cause of ID.

An un-blinded study[19] and a recent double-blind controlled study[21] documented oral iron treatment reduces RLS symptoms, particularly for those with lower ferritin values. Another study failed to include adequate numbers of patients with low serum ferritin and accordingly did not find any benefit from oral iron treatment.[22] Thus when peripheral iron stores are correctly measured and found to be low, treatment with oral iron reduces RLS symptoms. It is important to note that there is no substance other than iron that has been conclusively shown in some patients to both cause RLS when abnormal and cure RLS when the abnormality is corrected. Some limited evidence indicates that for some patients the dopaminergic system follows a similar although somewhat less dramatic pattern. Levodopa[23,24] and dopamine agonists[25,26]significantly relieve and dopamine antagonists[27] exacerbate RLS symp-

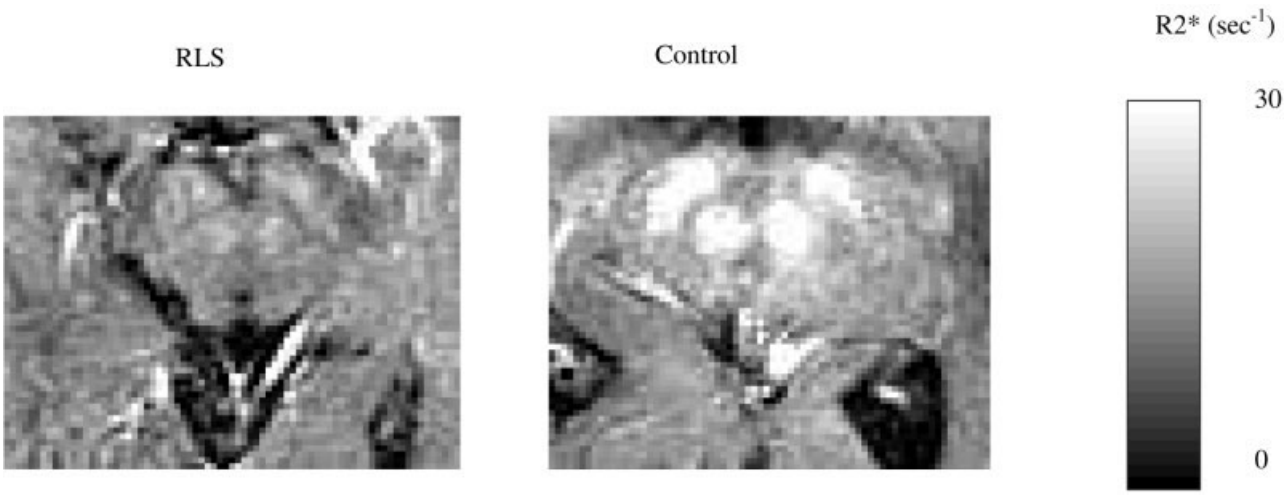

FIG. 2. R2* images in a 70-year-old patient with RLS and a 71-year-old control subject. Much lower R2* relaxation rates are apparent in the RLS case in both red nucleus and SN. (Reproduced with permission from Allen, et al., Neurology, 2001, 56, 263–265, © Lippincott Williams & Wilkins).

toms. The data for both the adverse and positive effects of dopamine on RLS, are less clear than that shown for iron, nonetheless this convergence of similar strong response to change suggests a possible iron–dopamine connection.

The recognition both of RLS as a CNS disorder and the strong link between peripheral iron status and RLS symptoms supports the following postulate: *Patients with RLS have marginal CNS iron status that: can become insufficient when deprived of normal access to adequate peripheral iron or may be insufficient even with normal access to adequate peripheral iron.*

The recognition of the strong effects on RLS symptoms of the changes in the dopaminergic system similar to the effects of changes in iron led us to further postulate: *the change or reduced CNS iron status produces RLS symptoms largely through its effects on the dopaminergic system.*

This postulate yields an important corollary: *Dopaminergic system abnormalities producing RLS symptoms will be included in those produced by brain ID.*

This enables research into the nature of the dopamine abnormalities of RLS using in vitro and in vivo techniques evaluating effects of ID on the dopaminergic system. The implications of this corollary are reviewed later after first evaluating the primary postulate of brain iron insufficiency in RLS.

Brain Iron Status and RLS

Brain iron concentrations differ dramatically both by areas of the brain and with normal aging.[28] Magnetic resonance imaging using special sequences provides measures of the iron concentrations in specific brain regions.[29-31] The putative iron–dopamine connection focuses interest on the dopamine producing areas with high iron-concentration and particularly the substantia nigra (SN). Two MRI studies using the GESFIDE sequence have found significantly reduced iron content in the SN more marked for those patients with RLS whose symptoms started before they were 45-years-old ("early-onset RLS"). In both of these studies the MRI measurement of nigral iron concentration correlated inversely with clinical ratings of disease severity.[32,33] An independent study using B-mode transcranial ultrasound imaging showed marked hypoechogenicity for the nigra area in patients with RLS compared to controls consistent with reduced nigral iron.[34] Thus, we have independent studies using different methods indicating nigral iron compromise in patients with RLS. Reduced nigral iron in patients with RLS compared to controls can be clearly seen in Figures 2 and 3.

CSF analysis provides another approach to assessing CNS iron status. Two CSF studies, one in Japan[35]and the other in the United States,[36] reported patients with RLS compared to matched controls had significant decreases in CSF ferritin and increases in CSF transferrin for samples obtained in the morning (about 10 A.M.). In these studies, in contrast to the CSF, the serum iron measures of ferritin and transferrin did not differ significantly between RLS and controls. Curiously one study of CSF collected in the evening (around 10 P.M.) showed a significant CSF ferritin decrease compared to controls for patients with RLS with early-onset of symptoms but not for those with late-onset (after age 45) of RLS symptoms.[37]

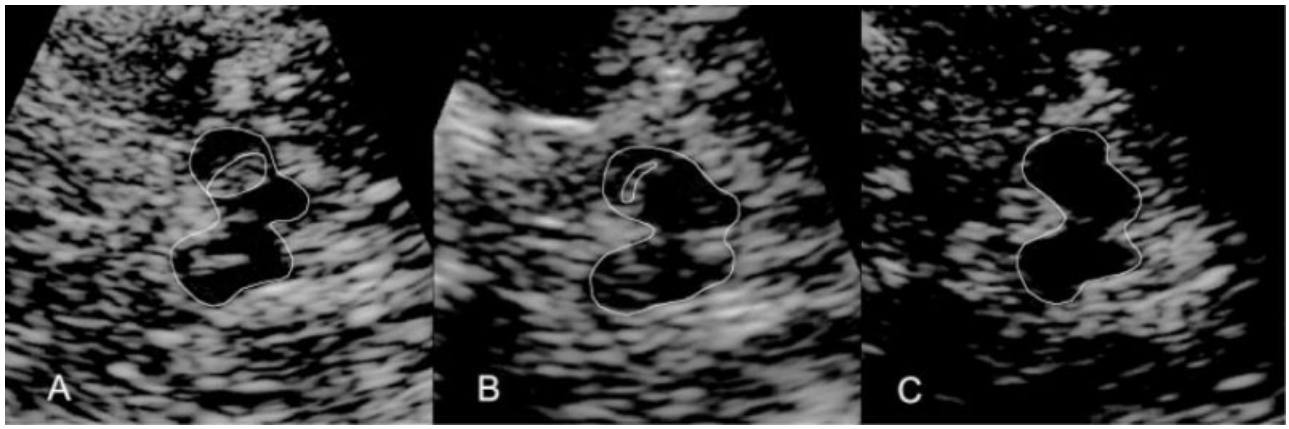

FIG. 3. Ultrasound showing decreased echogenicity in the midbrain area of the nigra for RLS (**C**) compared to control (**B**). (Reproduced with permission from Schmidauer, et al., Ann Neurol, 2005, 58, 630–634, © Wiley-Liss).

Circadian changes in CNS iron status appear to be a significant factor in RLS.

An important aspect of the CSF studies comes from examining the relation of CSF to serum ferritin. The recognized positive correlation between these two measures was found to be significant in both studies for controls and also for patients with RLS.[35,36] The patients, however, showed a much lower slope for this relation indicating some impairment reducing the usual gain in CNS iron status with improving peripheral iron status. The normal CSF ferritin shown by the control patients with a normal serum ferritin of 100 μg/L might only occur for patients with RLS with serum ferritin over 400 μg/L, generally considered to be abnormally elevated. (see Fig. 4).

The imaging and CSF results are consistent with the clinical findings: *RLS has both reduced brain iron status and also impaired ability to gain brain iron from peripheral iron.* The impaired gains in brain iron from peripheral iron may occur from problems with either transport to the brain or the brain's ability to hold onto iron or both of these.

Autopsy studies from early-onset (before age 45) patients with RLS provide strong confirmation of the brain ID in RLS and also give an interesting picture of the nature of this deficiency. Examination of stained sections from the SN of patients with RLS and matched controls showed decreased iron and H-ferritin along with increased transferrin.[38] The decrease in H-ferritin was particularly marked. There were two somewhat unexpected findings from these initial studies. L-ferritin was not decreased but it was concentrated in different cell types than normal. Since the majority of iron in the brain is stored in the oligodendrocytes, L-ferritin is normally substantially higher in these cell types. In RLS brains, however, astrocytic cells that normally have only mild L-ferritin staining had the major proportion of L-ferritin compared to surrounding tissue.[38] The mechanism behind this redistribution of iron to astrocytes rather than oligodendrocytes remains unexplained. This could, however, explain the failure of iron although present to be adequately available for the neuron. It also raises concerns about the interpretation of MRI findings regarding RLS brain iron status. A failure to find a change in iron in a brain region does not exclude the possibility of abnormal iron redisposition and thus abnormal utilization.

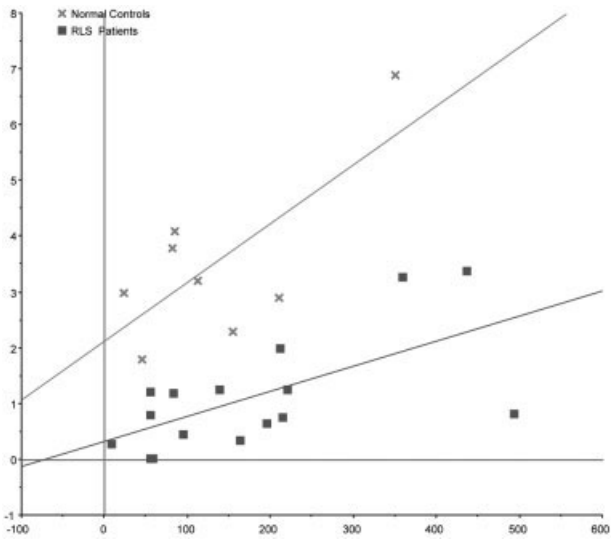

FIG. 4. Linear regression plots of serum ferritin and CSF ferritin for patients with RLS and normal controls from 10 A.M. samples. (Taken with minor alterations from Earley, et al., Neurology, 2000, 54, 1698–1700, © Lippincott Williams & Wilkins).

The second unexpected finding was a decrease in transferrin receptor.[38] An increase should occur with ID. Transferrin receptor status is post-transcriptionally regulated by iron regulating proteins IRP1 and IRP2. These were evaluated in a study used microlaser capture techniques to isolate neuromelanin cells of the SN. Examination of the homogenates of only the isolated neuromelanin cells showed that RLS compared to controls had increased IRP2 consistent with cellular ID but also an unexpected decreased IRP1 both in its active and aconitase form.[39] The marked decrease in IRP1 may explain at least in part the decreased transferrin receptor possibly limiting cellular iron access and contributing to the brain ID or even to reduced transport across the blood brain barrier. The decreased IRP1 may be secondary to the lack of availability of iron or reflect a primary pathology of RLS.

The homogenates of the laser-captured neuromelanin cells also showed the expected decrease in H-ferritin with no change in L-ferritin. In addition two iron transport proteins DMT1 and ferroportin were significantly decreased.[39]

Overall, the early-onset form of RLS occurs with abnormal iron regulation in the brain that appears likely to cause the RLS symptoms rather than the reverse. Whether or not this also occurs in some late-onset patients with RLS perhaps to in a different form or degree remains to be determined.

IRON–DOPAMINE CONNECTION

If, as seems incontrovertible, RLS links closely to iron status then how does reduced iron status produce the RLS symptoms? If, as was noted above, changes in dopamine status also change RLS symptoms, then, as postulated above, iron abnormality may produce these dopaminergic changes that produce the RLS symptoms. But, how does reduced iron change the dopaminergic system? It should be noted that answering this question may reveal details of the dopamine pathology in RLS that would otherwise be hard to directly measure for patients with RLS. The evaluation of the effects of iron on the dopaminergic system opens a wide range of studies. The iron–dopamine relation can be studied using in vitro cell models with differing degrees of iron chelation and also with in vivo animal models using either dietary induced ID or natural strain variation in brain iron.

In vivo studies using dietary induced ID in animals provides an experimental model to study effects of iron changes on the dopamine system. Dietary ID for Sprague-Dawley rats starting post weaning (e.g. day 21) and continuing for 2 to 4 weeks produced, compared to controls, 30 to 50% lower brain iron with increased transferrin and decreased ferritin.[40] The iron concentration decreases varied between brain regions. The iron decreased by 60% in the ventral midbrain which contains the SN, 30% in he caudate-putamen, and 20% in the nucleus accumbens.[41] ID lead to a decrease in striatal D1 and D2 receptors. The D2 receptor density correlated positively with striatal iron ($r = 0.91$) while the D1 receptor density showed no correlation with iron.[41] The striatal dopamine transporter (DAT) density decreased by 30% in the caudate putamen and 20% in the nucleus accumbens for the same dietary ID protocol.[42] Microdialysis assessment of striatal dopamine again using a similar ID protocol showed a 53% increase in extracellular dopamine compared to controls that occurred for the time period from the end of the inactive (light) through the first part of their active (dark) periods. Other time periods were not analyzed. More recent studies found an increased in the nigral tyrosine hydroxylase and increase in its phosphorylated form with dietary ID.[43] Tyrosine hydroxylase, particularly in its phosphorylated form, is also increased with iron chelation of PC12 cells[43,44]. Thus, in vitro and in vivo data provide further evidence for increased dopamine production contributing to the increased extracellular dopamine.

Finally, one interesting study looking at brain homogenates of dietary ID adult rats compared to controls found decreased Thy1, a protein commonly expressed on the surface of neurons that may have an important role for synaptic function.[45]

In summary, these in vivo and in vitro studies indicate that ID reduces striatal D2R and DAT and increases striatal extracellular dopamine enhancing the amplitude of the normal circadian pattern. The increased extracellular dopamine could result from either increased DA production, decreased uptake of DA or both, but the increased Th and pTh suggests this is at least in part a result of increased DA production in RLS.

IRON–RLS SYMPTOM CONNECTION

ID provides an in vivo animal model expressing RLS behavioral phenotype.

Although the sensory discomfort experienced by an patient with RLS cannot be directly evaluated in a non-human animal model, other aspects of the syndrome can be. In particular, the circadian pattern of activity should be altered reflecting a response to the circadian variation in the RLS symptoms of urge to move the legs. The Dietary ID animal model of RLS like RLS produces brain iron insufficiency but unlike RLS also produces systemic ID anemia. This limits the value of this model for evaluating most behavioral tests since the significant anemia in these animals dominates the animal's behavior

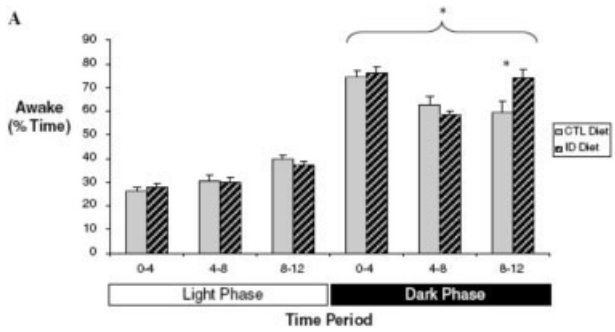

FIG. 5. Murine iron-deprived model of RLS: wake as percentage of each 4-hour period during baseline for iron-deprived and control mice (*$P < 0.05$). ANOVA confirmed a significant interaction between diet and time-of-day for the dark period with pair wise analyses showing significant increase in wake during the last 4-hour period. (Reproduced with permission from Dean, et al., Sleep Med 2006, 7, 634–640, © Elsevier Science).

and in particular would be expected to dramatically reduce overall activity levels. The relative circadian pattern of rest versus active period might, however, be changed with the urge to move reflected by a relative increase in activity during either the end of the active or in the inactive period. Youdim and Yehuda[46] had previously reported exactly this reversal of the normal circadian rhythm of activity with dietary iron deprivation. A more recent study by Dean et al.[47]used the standard post-weaning dietary iron-deprivation but used mice instead of rats and also produced a less severe systemic anemia (Hct as %RBC of 35–40 when compared with 16–18 in the other studies above,[41] controls Hct were 41–50). The wake time was increased relative to controls for the iron-deprived mouse but only in the last part of the active period.[47] (See Fig. 5) These data on the sleep-wake pattern of an animal model of RLS are consistent with the primary subjective complaint of sleep disturbance reported by patients with RLS. Both of these behavioral results from the iron-deprived rodent are consistent with the circadian pattern of RLS symptom expression with increased activity during the usual rest or inactive period and difficulty resting or being inactive as required for sleep in the times before the usual primary sleep period. These provide limited behavioral support for the ID model of RLS

THE IRON MODEL OF THE NEUROBIOLOGY OF RLS

We now have a remarkable confluence of results from in vivo, in vitro, and autopsy studies documenting ID effects on the dopamine system. The RLS pathology appears to involve abnormal cellular regulation of dopamine production leading to increased dopamine production and at least for part of the circadian cycle increased extracellular dopamine. The reduced DAT and D2R would reduce the information provided to the presynaptic cell regarding extracellular dopamine levels. This would lead both to abnormally increased dopamine production and also an abnormally increased circadian amplitude in the amount of extracellular dopamine. The decreased Thy1 might also contribute to altered dopamine function. In this view, the amplitude of the RLS circadian pattern for dopamine remains increased but its low point or trough may be decreased with its peak levels increased relative to normal. Postsynaptic receptors may not adequately adjust to such an abnormally large circadian variation producing abnormally decreased dopamine stimulation during the circadian trough of dopamine activity. This trough roughly corresponds to the period of RLS symptoms. Moreover, the large increase in dopamine stimulation at the end of the inactive and beginning of the active cycle corresponds to the "protected period" for RLS in the morning when few if any RLS symptoms occur. This morning period also corresponds with the times that patients elect to get up and not "sleep-in " despite short sleep times. This may reflect a general abnormally increased morning dopaminergic activation in the brain overcoming some effects of chronic insufficient sleep.

The in vitro data also, however, indicate possible abnormalities in other neurotransmitter systems such as adenosine and glutamate.[48] The iron model of RLS gives us the tools to examine dopamine and other abnormalities in animal and cellular models and to explore genetic factors.

IRON TREATMENT OF RLS

As noted earlier, oral iron has been documented to give some limited benefit to patients with RLS with lower serum ferritin. The question remains of whether or not a large increase in peripheral iron via IV delivery would provide sufficient available iron to overcome the limitations in making this available to brain tissue. If so, it might reduce brain ID enough to reduce RLS symptoms. There have been two open label trials documenting benefit from this type of treatment. Nordlander, over 50 years ago, conducted the first published trial of IV iron treatment of RLS and reported it produced complete and fairly lasting relief from RLS symptoms for all but one of 22 subjects treated.[2,3] Earley et al. recently did a careful evaluation of response to a single dose of 1,000 mg of IV iron dextran using both clinical ratings and objective measures of leg movements during the sleep period.[49] They reported that 6 of 10 patients with RLS had almost if not complete relief from all RLS symptoms for at least

2 weeks and for most patients the relief lasted longer than 2 months. This relief was documented both on subjective scales and also on the objective measure of leg movements. Double-blind placebo-controlled studies are currently being conducted. It is important to recognize that failure of IV iron treatment may occur, despite ID if the RLS impairment of the normal modes of brain iron acquisition and retention exceeds the capacity for correction through even a very large increase in peripheral availability of iron. The severity of the iron management impairment may also differ considerably between patients and correction by a large influx of peripheral iron may be possible only for those with less severe impairment who presumably have somewhat less severe symptoms. It may also be that the iron treatment works better if the higher levels of iron availability are maintained for several hours or even days to permit the brains impaired iron management continuous access to a rich supply of iron. Longer half-life iron formulations may therefore be needed to produce treatment benefits.

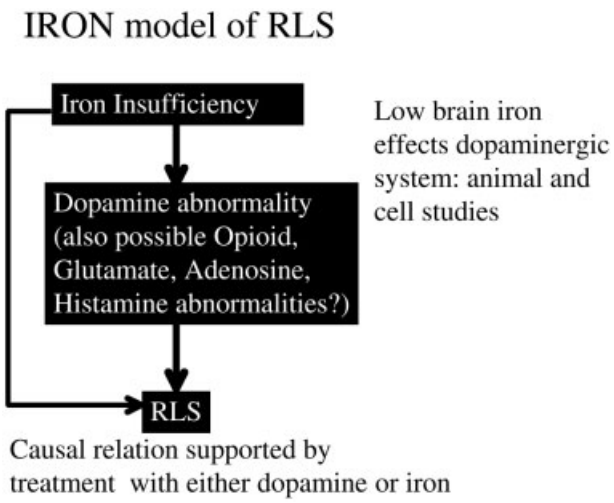

FIG. 6. The iron model of RLS. Brain ID creates an abnormality in the dopaminergic system that produces the RLS symptoms. The Brain ID may also create abnormalities in other neurotransmitter/neuromodulator systems such as the opioid, glutamate, histamine, and adenosine which may also produce some of the RLS symptoms. This model does not limit the effects to any one specific brain region, although the effects may be more easily detected in some larger and more studied systems (e.g. nigrostriatal system for dopamine).

IRON AND OTHER CAUSES OF RLS

A common and complex syndrome like RLS is unlikely to have one cause. Any number of factors other than iron insufficiency might produce dopaminergic or other abnormalities that could produce RLS symptoms. Theories abound, but there are only meager data showing actual clinical relations of factors other than iron and dopamine to the occurrence of RLS symptoms. Some data indicate that RLS occurs more with the use of SSRIs,[50] with some forms of neuropathy[51-53] and possibly with rheumatoid more than osteoarthritis arthritis[54]. However, even rheumatoid arthritis involves problems with iron management and the relation to RLS may be secondary to this iron problem.[55] Whether or not the other conditions associated with RLS involve iron problems or interact with iron status of the patient has not been evaluated. It, however, seems likely that RLS will have multiple determinants some of which may not involve iron at all. That iron insufficiency probably does not cause all of RLS does not reduce its importance as one major cause of RLS that provides us with basic information about RLS pathology. It may also interact with these other determinants of RLS.

The iron abnormalities consistently found in RLS studies appear likely to be pervasive involving not only the SN but probably in various degrees other dopaminergic and other neurotransmitter systems. (see Fig. 6) The neural areas affected by RLS are similarly likely to involve more than one part of the nervous system. Determining anatomical locations for RLS may tell us less than looking at what disrupts the neural function; thus a strength of the iron model of RLS is that it would differentially affect diverse parts of the brain consistent with evidence for RLS pathology in several brain areas including the tuberoinfundibular,[56] A11-spinal,[57] thalamic,[58,59] and nigrostriatal systems.[38]

SUMMARY: THE ROLE OF IRON IN RLS

We can no longer say the pathophysiology of RLS is unknown. Brain iron and particularly iron in the SN is reduced in RLS, particularly for the early-onset (before age 35–45) phenotype that tends to have greater familial occurrence. Moreover, reducing iron availability to the brain by any change in peripheral iron increases both the chance of developing RLS and also the severity of RLS. Replacing iron reduces RLS symptoms and in some cases completely corrects the problem. Thus, an abnormality in iron management compromising brain iron status causes RLS. There are undoubtedly other independent causes of RLS, but this is a major cause of the disorder. Other factors may interact with the iron pathology to either enable or protect from RLS expression, but the iron pathology remains one that is central to the disease process in RLS.

The iron model of RLS provides powerful research tools for assessing the neurobiology of RLS. Since iron insufficiency causes RLS, its neurobiological effects are likely to include the ones that cause RLS symptoms. In particular, it may cause RLS symptoms through its ef-

fects on the dopaminergic and other neurotransmitter systems (see Fig. 6). It maybe, however, that only a subset of the biological effects of ID causes RLS, particularly since iron is probably only one of the causes of RLS. Nonetheless the iron-model should reveal essential abnormalities causing RLS symptoms. The in vivo animal models of ID also produce some of the behavioral phenotypes of RLS and certainly provide a good biological model of RLS. Both the in vivo and in vitro models of ID reveal dopamine abnormalities that have been largely confirmed in autopsy studies of brains from patients with RLS. These critical studies serve to confirm the iron model of RLS and also provide a biological basis for the dopaminergic treatment of RLS. Moreover, the ID as a biological model of RLS permits evaluating involvement of other neurological systems including but certainly not limited to adenosine and glutamate. Evaluating these in the iron models and also autopsy or clinical studies would further confirm the iron model of RLS and might indicate targets for developing or understanding mechanisms of nondopaminergic treatments for RLS. These studies will likely inform about the neurobiology not only of RLS but also of RLS related states such as the interesting brain state of quiet resting, a state normally required before sleep but one that provokes RLS symptoms. The iron model also provides some alternative ways forward for genetic studies both using in-bred strains of mice and also possible endophenotypes.

Recognizing the central role of iron pathology in RLS, however, requires some refocusing of our attention. We need to study the abnormalities in neurological circuits and neurotransmitter systems produced by the ID as an indication of the neurobiology of RLS. These important studies serve to confirm the role of iron and indicate biological bases and targets for treatment. But, we also must not forget to evaluate the fundamental iron problem of RLS. We need to better determine the iron management pathology in RLS. Perhaps the iron pathology itself can be treated.

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