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Review Article

Proposed Theories of Restless Legs Syndrome Pathophysiology-A Critical Review

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Abstract

Introduction: Restless leg syndrome is a common sleep related disorder leading to increased continuous urge to move limbs from 4 pm to 4 am. Multiple theories have been proposed with respect to restless legs syndrome like role of pontine infarction in restless legs syndrome. Different groups studied multiple neurotransmitters like gamma-Aminobutyric acid concentration in different parts of brain and linking it to restless legs syndrome as well.

Methods and Results: In current review, critical analysis of individual studies was conducted evaluating credibility of experiments leading to a final opinion pertaining to restless leg syndrome pathophysiology. Possible overlaps among different mechanisms were also identified to provide robust conclusion.

Conclusion: Strong evidence exists which differentiates the rodent and non-human primate/human neuroanatomy specially pertaining to certain types of dopamine receptors. A11 dopaminergic neurons pathway links to earlier evidence and provides a strong base to further study the concept. In future more, robust animal models pertaining to restless legs syndrome and double-blind placebo controlled clinical trials need to be conducted to validate these theories.

Keywords: *Restless Leg Syndrome; Sleep Disorders; Restless Legs Syndrome Treatment; Restless Legs Syndrome Pathophysiology*

Introduction

Restless leg syndrome is a common sleep related disorder leading to increased continuous urge to move limbs from 4 pm to 4 am. This clinical condition has significant interference in sleep and has drastic implications on health [1]. Based on consensus, International Restless Legs Syndrome Study Group, four essential criteria have been recommended for diagnostic purpose: a) desire to move limbs associated with paresthesia or dysesthesia, b) improved condition secondary to movement c) worsening of symptoms with no movement and alleviation of symptoms temporarily on motion d) worsening of condition in evening and at night. Scientific groups have proposed two types of presentation of restless legs syndrome: early onset disease (less than 45 years) and late onset disorder. Former type is linked to genetic predisposition and disease progression is at slow pace, whereas late onset type has faster progression [2].

Multiple theories have been proposed with respect to restless legs syndrome. In this context [3] studied role of pontine infarction in restless legs syndrome. Another group studied multiple neurotransmitters like gamma-Aminobutyric acid concentration in different parts of brain and linking it to restless legs syndrome [4]. Role of peripheral neuropathy as pathophysiological factor in restless legs syndrome patients in a prospective study was evaluated by another group [5]. Iron metabolism derangement and its concentration in blood and blood brain barrier also investigated for its role in restless legs syndrome. Some studies evaluated role of iron and folate during pregnancy and its association with restless legs syndrome [6,7]. Theories have also been proposed indicating role of Opioid system in restless leg syndrome [8].

Conner and team conducted a study evaluating association of deranged myelination of neurons and restless legs syndrome symptoms [9]. Anderson focussed on peripheral neuropathy as pathophysiological cause complicating restless legs syndrome presentation. Multiple genetic loci (19 till now) have been proposed which have role in restless legs syndrome [10]. A11 dopaminergic pathways have also been proposed in animals like rodents and non-human primates [11,12].

In current review, critical analysis of individual studies was conducted evaluating credibility of experiments leading to a final opinion pertaining to restless legs syndrome. Possible overlaps among different mechanisms were also indentified to provide robust conclusion.

Different proposed theories with respect to restless leg syndrome

In a Clinical study role of Pontine infarction as causative factor of Restless leg syndrome was evaluated. Study comprised of 5 clinically diagnosed cases of Stroke with Pontine infarction. Sleep evaluation done based on Epworth Rating Scale. Follow up period was 12 - 42 months. There was no history of restless legs syndrome, Diabetic neuropathy, anemia, sleep apnoea and other neurodegenerative disorders. All patients received standardized treatment for Pontine infarction and had significant clinical improvement after 2 weeks, however restless legs syndrome symptoms were not relieved completely, and patients were put on Dopamine agonists (3 of the 5 patients) based on severity of restless legs syndrome symptoms.

It was observed that patients receiving dopamine agonists had improvement in restless legs syndrome symptoms within 3 months of treatment while remaining 2 did not improve on restless legs syndrome symptoms. Based on statistical analysis (T test paired -SPSS), there was significant improvement in restless legs syndrome score ($p = 0.035$), baseline (20.60 ± 10.04) and post treatment follow up (5.60 ± 8.76) [3].

This Clinical case study provides evidence of cerebral infarction involvement in initial restless legs syndrome, however recovery from infarction did not improve symptoms of restless legs syndrome and patients (3 out of 5) put on Dopamine agonist had significant improvement. In MRI findings, it was observed that all 5 patients' infarction area was rostral pons which is linked to pyramidal pathways of brain hence evidence that RES restless legs syndrome can be disease of Central Nervous system. Current study although involved only 5 subjects, inclusion and exclusion criteria was stringent. Diabetic neuropathy cases and peripheral neuropathy cases were ruled out with help of NCV studies. Patients with history/family history of restless legs syndrome were also excluded. Assessments were based on International restless legs syndrome Rating Scale and Epworth Rating Scale. Tesla MRI was used to localize brain lesions. Key drawback of the study was very small sample size.

Winkelman studied multiple neurotransmitters gamma-Aminobutyric acid concentration in different parts of brain like anterior cingulate cortex, cerebellum and thalamus of study subjects ($n = 18$) restless legs syndrome and $n = 18$ controls). For purpose of evaluation of neurotransmitters levels Proton magnetic resonance spectroscopy (4 Tesla) and technique of MEGA was used. They also used Actigraphy for five nights to evaluate movements of restless legs syndrome. Polysomnography was also performed for single night. Pittsburgh Sleep Quality Index and restless legs syndrome were also recorded. Study results show no significant difference between restless legs syndrome and control arms with respect to concentration of gamma-Aminobutyric acid neurotransmitter in areas of brain studied. Major finding of

study was correlation between gamma-Aminobutyric acid and corresponding leg movements (based on actigraphy) in areas of Thalamus (Figure 1) and Cerebellum (Figure 2).

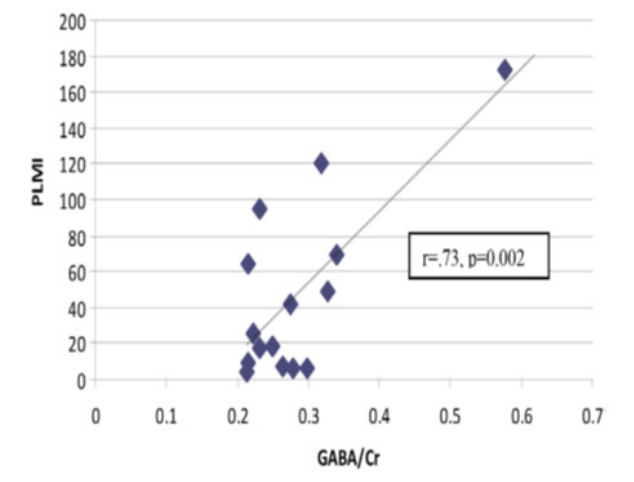

Figure 1: *PLM Index showed a strong positive correlation with GABA concentration in Thalamus (r = .73 at p = 0.002) [4].*

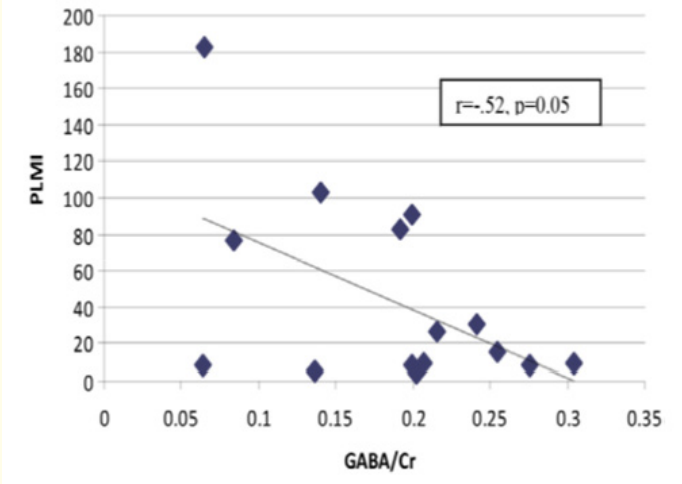

Figure 2: *PLM Index showed a strong negative correlation with GABA concentration in Cerebellum (r = -.52 at p = 0.05) [4].*

This study investigated concentration of various neurotransmitters in patients with diagnosed restless legs syndrome and control group. Though some correlation patterns were observed in Thalamus (Figure 1) and Cerebellum (Figure 2) with respect to gamma-aminobutyric acid neurotransmitter, there was no significant difference in restless legs syndrome and control group. This is major drawback of this study and hence relationship between gamma-aminobutyric acid neurotransmitter and restless legs syndrome can't be established.

Shukla investigated role of peripheral neuropathy as a pathophysiological factor in 57 restless legs syndrome patients in prospective study using heat sensation, nerve conduction velocity studies and electrode based sympathetic skin response. It was observed that in all patients, nerve conduction velocity test was normal. Similarly, other parameters were also not supportive of major peripheral neuropathic involvement. The study however indicates involvement of small peripheral nerves which seems to be more of a comorbid factor rather than pathophysiological cause. This study provides an indirect evidence of involvement of Central Nervous system rather than involvement of peripheral nervous system [5].

Study comprising of 24 restless legs syndrome diagnosed patients and control group evaluated restless legs syndrome symptoms secondary to iron deficiency. For purpose of Iron derangement evaluation fasting lymphocytic levels of ferritin, trans-ferritin, transferrin receptor and divalent metal transporter 1 protein were taken along with other biomarkers (Table 1 and Table 2) [6].

	Number	Age (years)	Hemoglobin (g/dl)	Ferritin (mcg/l)	TIBC	%Sat
Control	25	60.0 ± 1.7	13.5 ± 0.17	55.1 ± 6.5	318 ± 8.2	27.1 ± 1.8
RLS	24	58.3 ± 2.6	13.9 ± 0.16	52.8 ± 5.1	321 ± 6.5	27.8 ± 1.2
t, p	0.5, 0.59	1.7, 0.10	0.3, 0.78	0.3, 0.78	0.3, 0.78	

Table 1: *The Number Subjects Per Group, the Control and RLS Group Means ± Standard Errors of Age and of the Common Measures of Systemic Iron Status, and the Corresponding T-Score and Statistical Probability (P) [6].*

Data are presented as mean ± SEM. Hgb refers to hemoglobin, TIBC: Total Iron Binding Capacity; %Sat: Percent Iron Saturation.

Lymphocyte Measures of Iron Status	Serum Measures of Iron Status							
	RLS Group				Control Group			
	Hemoglobin	Ferritin	% Sat	TIBC	Hemoglobin	Ferritin	% Sat	TIBC
H-ferritin	0.05	0.06	0.14	0.16	0.05	0.05	0.14	0.02
L-ferritin	0.12	0.08	0.18	0.01	0.12	0.02	0.17	0.03
Transferrin	0.21	0.39	0.18	0.20	0.15	0.00	0.23	0.04
TfR	0.12	0.04	0.05	0.21	0.18	0.03	0.07	0.10
DMT1	0.13	0.05	0.01	0.01	0.05	0.03	0.20	0.20
Ferroportin	0.06	0.03	0.21	0.01	0.19	0.03	0.04	0.32

Table 2: *The Pearson Correlations Between Serum and Lymphocyte Measures of Iron Status [6].*

TfR refers to transferrin receptor; DMT1: Divalent Metal Transporter 1 Protein.

The cellular levels of iron have inverse relation with to transferrin receptor and divalent metal transporter 1 protein concentration. restless legs syndrome subjects had higher levels of transferrin receptor and divalent metal transporter 1 protein compared to controls indicating increased utility of iron in restless legs syndrome and hence increased turnover. Similarly, ferroportin (an iron transporter) was increased in restless legs syndrome group indicating a greater number of iron molecules moving out of lymphocytes.

Current study focuses on iron metabolism derangement as a pathophysiological factor of restless legs syndrome. Study provides an idea how Lymphocytic iron metabolism is impacted in restless legs syndrome though there was no significant difference found in iron markers of restless legs syndrome and control group. Major drawback of study is early cases of restless legs syndrome were chosen. Study provided evidence on overall increased turnover of iron in restless legs syndrome cases, however chronic restless legs syndrome patients

may have different levels of lymphocytic iron markers and serum levels of ferritin. Hence further studies are required to evaluate iron derangement in chronic restless legs syndrome patients establishing iron deficiency as pathophysiological factor leading to restless legs syndrome.

Connor and his colleagues studied brain iron metabolism in autopsy cases of restless legs syndrome patients. Histological samples of Choroid plexus 14 restless legs syndrome patients and 18 normal controls were evaluated using immune-histological validated methods. In restless legs syndrome patients there was decreased staining of iron and ferritin in choroid plexus histological samples (Figure 3) [7].

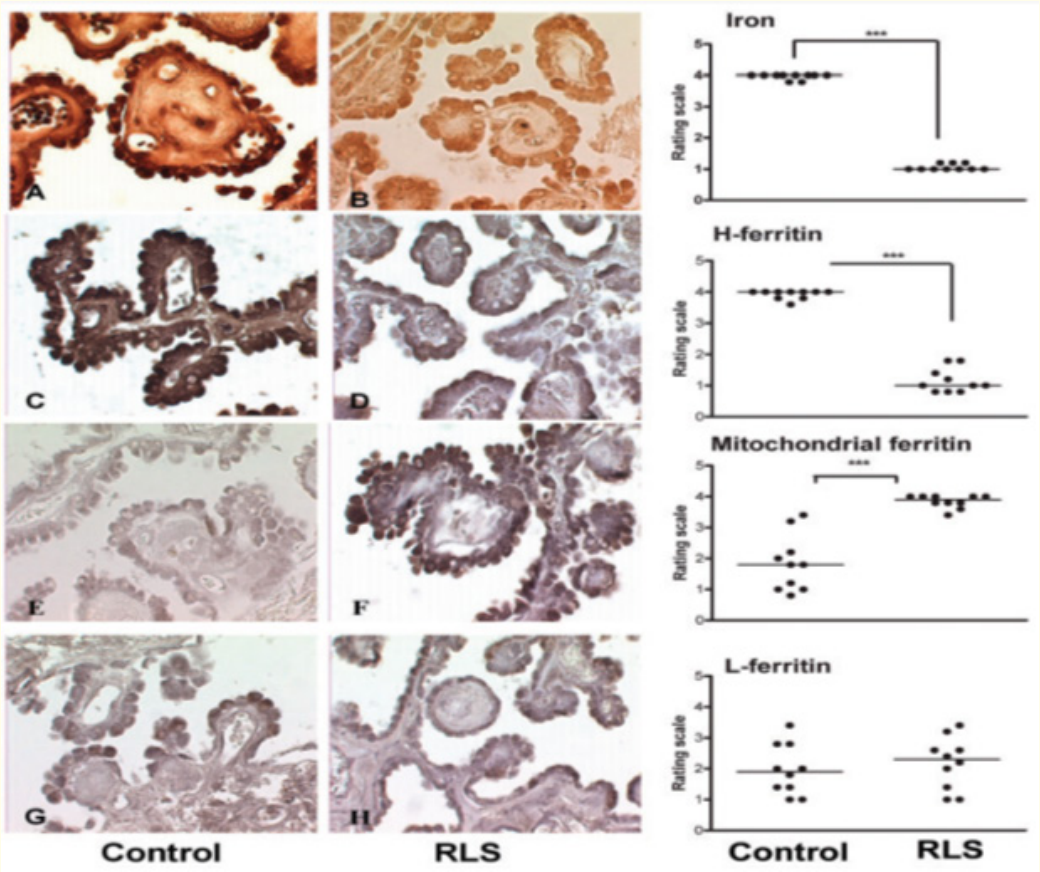

Figure 3: *Demonstrates significant reduction in Iron and Heavy chain ferritin staining in RLS patients choroid plexus tissue as compared to controls (p < 0.001) ([7].*

They further investigated microvasculature of choroid plexus using immunoblot methodology (11 restless legs syndrome cases and 14 normal controls autopsy). Levels of ferritin and transferrin were significantly reduced in restless legs syndrome arm as compared to control arm (Figure 4).

Current study provides evidence pertaining to iron metabolism's role in restless legs syndrome symptoms. Iron regulatory molecules had significant lower levels in choroid plexus microvasculature of restless legs syndrome autopsy cases as compared to controls. Further, concept of blood brain barrier and endothelial cells being reservoir of iron regulatory molecules is being highlighted by study.

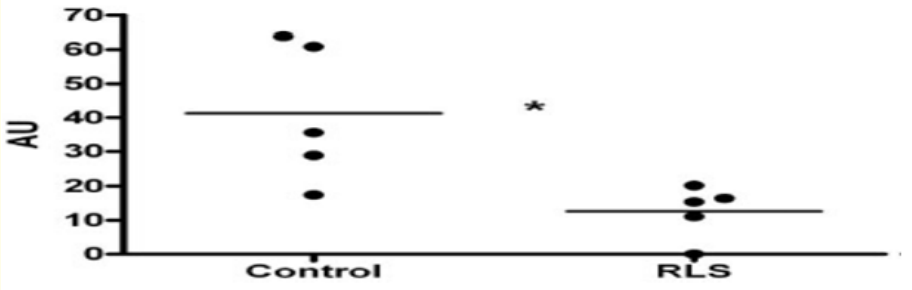

Figure 4: *Depicts binding activity of Iron regulatory protein in RLS and control group (Brain microvasculature was assessed p < 0.05) [7].*

In a longitudinal prospective study (n = 7) role of iron and folate deficiency as pathophysiological factor in restless legs syndrome during pregnancy was evaluated. Concurrent blood sampling was done to get assessment of iron and folate levels. Prevalence of restless legs syndrome symptoms here increased by 23% in 3rd trimester. There was significant decrease in folate levels by end of 3rd trimester compared to iron levels in all subjects. This study provides evidence regarding folate deficiency involvement in restless legs syndrome rather than iron deficiency. Key drawback of study was small sample size and randomized controlled clinical trial are required to further validate this concept. Further, results obtained cannot be generalized to pregnant women population [13].

Spiczak investigated role of opioids in restless legs syndrome using positron emission therapy technique in patients with restless legs syndrome (n = 15) and healthy subjects (n = 12). For positron emission tomography imaging [11C] diprenorphine (opioid Ligand which radioactive) was used. Pearson's correlation analysis between [11C] diprenorphine binding with different parts of brain including Amygdala (r1 = -0.837 right with p value < 0.001, r^2 = -0.700 left with p value = 0.005), Anterior Cingulate Gyrus (r = -0.709 with p = 0.005), Right Thalamus (r = -0.751 with p = 0.005) and Orbitofrontal Cortex (r = -0.697 with p = 0.006) (Figure 5) [8].

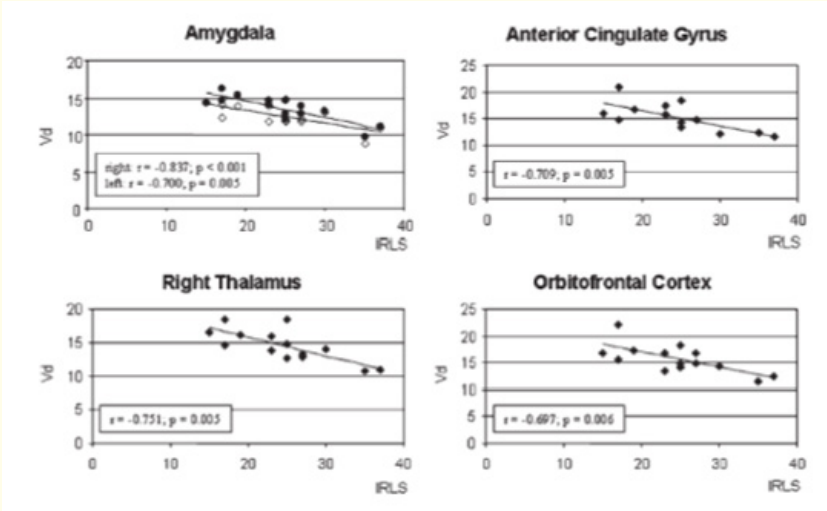

Figure 5: *Negative correlations were observed between [11C] diprenorphine binding with different parts of brain including Amygdala, Anterior Cingulate Gyrus, Right Thalamus and Orbitofrontal Cortex (All values statistically significant) [8].*

This study supports theory of release of endogenous opioid substances in patients with severe restless legs syndrome symptoms as compared to controls. It was found that binding capacity of radioactive opioid substance was significantly negatively correlated with areas of brain primarily responsible to carry on functions like pain relief based upon opioid action sites/receptors. [11C] diprenorphine not binding to sites of action gives an indication pertaining to non-availability of receptor sites to bind as these are already blocked with endogenous opioid substance suggesting opioid system involvement being a reactive mechanism leading to endogenous release of opioid substances in response to severity of restless legs syndrome symptoms rather than actually causing pathology.

Connor and his team conducted study evaluating association of deranged myelination of neurons in patients (n = 11 restless legs syndrome patients and n = 11 matched controls) with restless legs syndrome based on autopsy and brain tissue evaluation for multiple myelin expressing proteins using western blot techniques. Myelin basic protein and proteolipid protein are key indicators of decreased myelin production. Conner's group observed significant reduction (25% p value = 0.05) in levels of Myelin basic protein, proteolipid protein and other factors responsible for expression of myelination of neurons. These levels were further correlated to Iron levels in brain indicating decreased myelination process involving mechanisms secondary to iron deficiency. They further validated concept via functional imaging techniques in 23 restless legs syndrome patients and compared it to 23 normal individuals. Decrease in volume of white matter in areas of brain like anterior cingulum, corpus callosum and precentral gyrus was observed (Figure 6) [9].

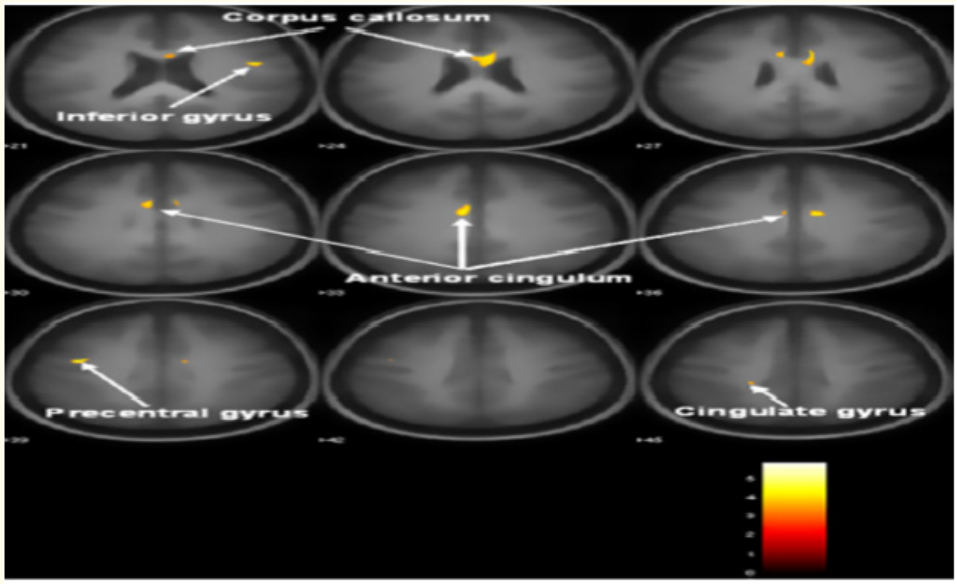

Figure 6: *Voxel based functional imaging indicating decreased white matter in anterior cingulum, corpus callosum and precentral gyrus in patients with restless leg syndrome (n = 23) when compared with similar normal individuals (n = 23). Volume reduction of White matter was although less in quantum however statistically significant (p < 0.001).*

Current study provides statistically significant results pertaining to demyelination as pathophysiological factor in restless legs syndrome patients, however demyelination process itself seems to be linked with iron reservoirs in brain and hence linking demyelination with iron deficiency in restless legs syndrome patients.

Anderson conducted retrospective study in restless legs syndrome patients with comorbid peripheral neuropathy (n = 42). Patients underwent surgical procedures (common and superficial fibular nerves decompression) for management of peripheral neuropathy. Pre

and post surgical procedure (15 weeks after intervention) symptomatic assessment was done based on Visual analog scale comprising symptoms reporting from 0 - 10 range. Results indicated significant reduction ($p < 0.01$ for all Visual analog scale aspects taken into account) in symptoms of restless legs syndrome post-surgical decompression of nerves (Figure 7) [14].

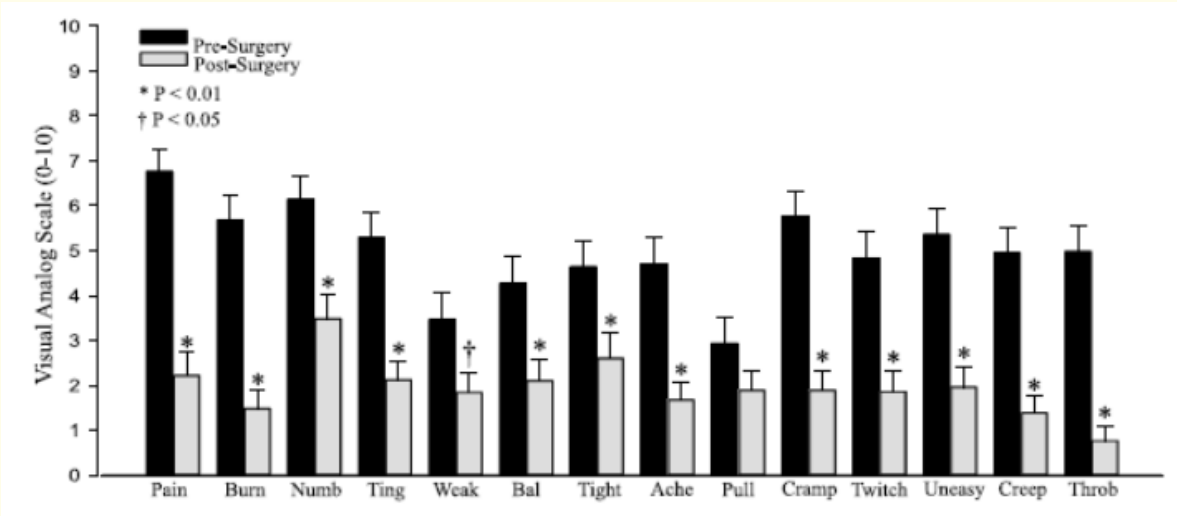

Figure 7: *Diagram depicts pre surgical (black bars) and post-surgical (grey bars) Visual analog scale scores in 42 patients with RLS. There was significant decrease in symptoms in all categories as per Visual analog scale [14].*

Further, change in total Visual analog scale score after surgery (Visual analog scale score post-surgery - Visual analog scale score pre-surgery) was correlated with pre-surgery Visual analog scale scores. Total Visual analog scale score change after 15 weeks of procedure was significantly negatively correlated with pre-procedural total Visual analog scale score at baseline was observed (Figure 8).

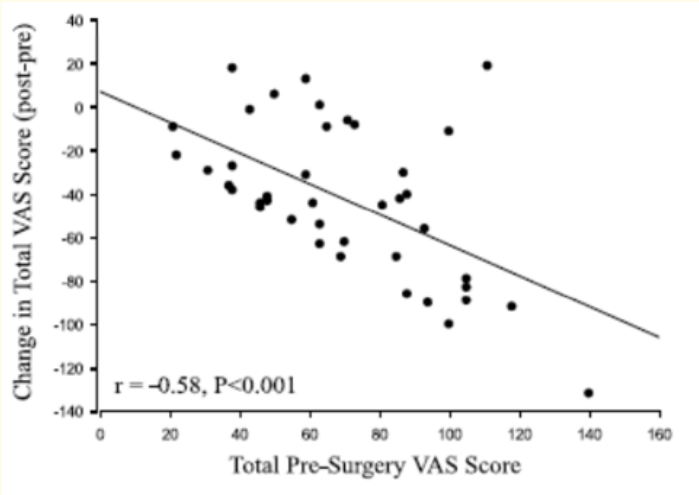

Figure 8: *Pearson's correlation between pre-surgery total Visual analog scale and Change in total Visual analog scale r = -0.58 and p < 0.001 [14].*

Current study provides limited evidence that Peripheral neuropathy and restless legs syndrome share at least some pathway in both pathologies. Though there was significant negative correlation between total Visual analog scale scores pre-surgery and VAS changes post surgery, but being a retrospective study, bias cannot be ruled out. Hence, it provides some background to investigate association between two pathologies and understand whether these are comorbid or restless legs syndrome is secondary to peripheral neuropathy.

In a metanalysis**,** identification of 13 new genetic risk loci for restless legs syndrome, based on genome wide associated studies (3 data sets) done on European population. Data collection done from 2003 - 2017 and total number of restless legs syndrome cases participated were 15126 and controls were 95725. The group did gene annotation and gene set enrichment analysis along with correlation between restless legs syndrome symptoms and genetic traipses 2 was most important risk loci identified (OR = 1.92 @ 95% CI). This analysis had great significance with respect to identification of 13 new genetic loci (Figure 9) related to restless legs syndrome symptoms and hence can be strong candidates responsible for pathophysiology of restless legs syndrome. All these loci have associated with activities like neurogenesis, synapse formation, axonal conduction and hence overall neurodevelopment, absence of which can lead to restless legs syndrome symptoms [10].

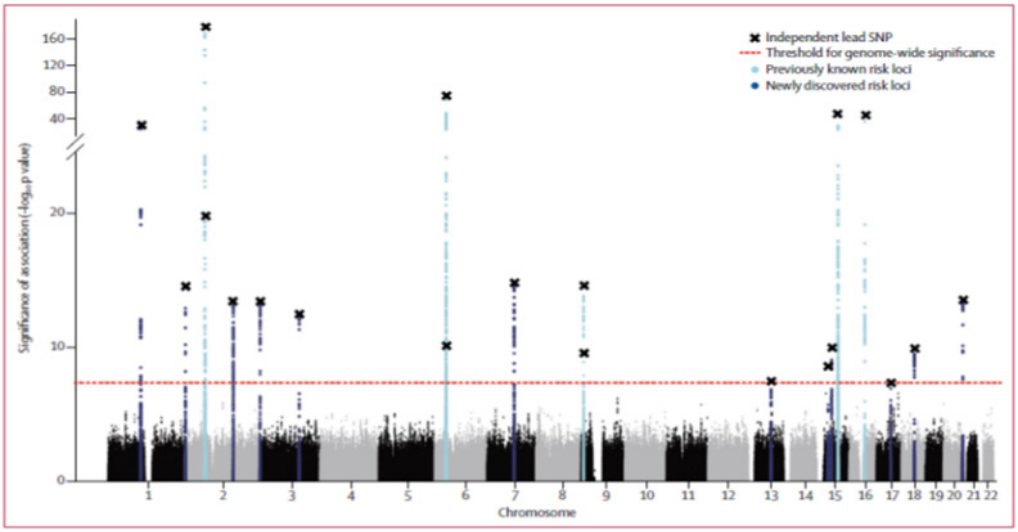

Figure 9: *Manhattan plot depicting 19 genetic loci (6 previously known and 13 new). At chromosome level 2 and 6, there is fusion of peaks of 2 loci and this is presented as single peak [10].*

The data further suggests requirement of studying neurodevelopment aspects in relation to pathophysiology of restless legs syndrome. Hence it calls for extensive animal and Clinical research to further validate findings. Overall 42 original articles were evaluated and sample for analysis was very high leading to statistically significant results. Table 3 provides list of 13 new genetic loci and their functional requirement.

Drawback was variation of restless legs syndrome symptoms severity and clinical presentation in all studies leading to overall variation in observations. Further studies are required which can link 13 new identified genetic loci in restless legs syndrome patients with corresponding phenotypes leading to consensus between scientific community and addressing neurodevelopment pathways involvement restless legs syndrome.

	Genes	Functions related to neurodevelopment
rs12046503	*NTNG1*	Presynaptic cell-adhesion molecule involved in synapse formation
rs10208712	*DCDC2C*	Encodes neuronal migration protein doublecortin, a member of the DCX protein family of cell-adhesion molecules; unknown function, but other members of the DCX family act in neuronal migration and axonal growth and have been linked to neurological and developmental disorders
rs113851554 rs1820989	*MEIS1*	Implicated in neurogenesis, specification of neuronal cell type, and establishing connectivity between neurons and their target field; binds HOX proteins of all paralogue groups, participates in controlling *HOX* gene expression
rs80319144	*PKP4*	Encodes the cell-adhesion molecule plakophilin-4, which serves as a scaffold for signalling complexes and plays a part in cell adhesion and neurite outgrowth
rs1848460	*CRBN, CNTN4*	Cereblon, encoded by *CRBN*, is the substrate receptor of a Cullin4a RING E3 ubiquitin ligase and regulates assembly and expression of calcium-activated potassium channels in the brain; contactin-4, encoded by *CNTN4*, is a cell-adhesion molecule with an important role in axon guidance, synapse formation, and neuronal network plasticity
rs17636328	*MDGA1*	Encodes MAM domain-containing glycosylphosphatidylinositol anchor protein 1, which is a trans-synaptic cell-adhesion molecule implicated in synapse development
rs10952927	*ZNF804B, ADAM22*	ZNF804B, which is highly homologous to ZNF804A, has been associated with schizophrenia and bipolar disorder; ADAM22 is a synaptic receptor involved in synaptic transmission and synaptic disorders
rs1836229 rs62535767	*PTPRD*	Related to functions in axon guidance and synaptogenesis, especially in the formation of excitatory synapses
rs340561	*DACH1*	Dach1 is a transcription factor acting as a neurogenic cell-fate determining factor
rs996064	*MEIS2*	Involved in neurogenesis and contributes to determination of dopaminergic-cell fate; binds HOX proteins of all paralogue groups and participates in controlling expression of *HOX* genes
rs111652004	*SEMA6D*	Involved in axonal pathfinding and signalling; exerts repulsive or attractive effects on axons, depending on the specific combinations of its main receptor with co-receptors; *SEMA6D* knockout mice show misdirection of proprioceptive axons and their associated oligodendrocytes in the dorsal horn, affecting proper synapse formation
rs45544231	*TOX3*	Implicated in neurogenesis, specification of neuronal cell type, and establishing connectivity between neurons and their target fields
rs12450895	*HOXB cluster* family	Assign positional identities to neurons along the rostrocaudal axis in hindbrain and spinal cord, which is crucial in the specification of neural subpopulations and their target cells; mouse models show the necessity of *Hoxb* genes for correct neuronal specification, migration, and circuit formation
rs365032	*MYT1*	Myt1 kinase is a transcription factor expressed in neural progenitor cells in the central and peripheral nervous systems; involved in neuronal differentiation by suppressing neural progenitor fate and promoting neurogenesis

Table 3: *List of 13 new genetic loci and their functions which can be proposed as pathophysiological factors leading to restless legs syndrome [10].*

Shen Q proposed an animal model for understanding restless legs syndrome. Model was based on dual concept of Iron deprivation in brain and diencephalic-spinal (A11) dopaminergic nucleus lesions induced in mice. A11 dopaminergic is subcortical system and linked with spinal cord along with certain circadian regions. Lesions in this region are proposed to be associated with restless legs syndrome. Shen combined two proposed pathophysiologies of iron deficiency and A11 dopaminergic lesions in mice and proposed same as possible animal model for understanding pathophysiology of restless legs syndrome and investigating various treatment options [11].

Total n = 80 mice (C57BL/6) of age 28 days after birth were divided into two groups of 40 each. In experiment one: on 28th day of age mice were divided into two subgroups, one group of 40 received regular iron diet and other group iron deficient diet. Parameters assessed at baseline and 1 month post iron deficient diet were: Weight recording, Serum iron concentration and behavioral aspects in both groups. It was observed that iron deficient diet group had significantly lower levels of serum iron (46 ± 2.4% @$p < 0.01$) after 1 month of experiment as compared to controls. After one month, follow up experiment done in which 40 mice were induced with 6-Hyroxydopamine lesions and assessment done for parameters like Weight recording, Serum iron concentration and behavioral aspects of mice after 1 month in both groups.

It was observed that mice with A -11 lesions had significantly ($p < 0.01$) low levels of brain, spinal cord and serum iron when compared to sham controls. Further iron diet deprived group which was later subject to A 11 lesions demonstrated further lower levels of iron in brain region ($p < 0.01$). In both experiments there was significantly ($p < 0.01$) increased loco-motor activities displayed by iron diet deprived and A 11 lesions mice group as compared to controls. With respect to weight and survival there was no significant difference observed in all the groups. All groups were given D1, D2 and D3 agonists and behavior of animals was observed. With D2 and D3 agonists there was significant decrease in aggression behavior and locomotor activities of both iron diet deprived and A 11 lesioned mice when compared to controls. On other hand, administration of D1 agonist led to increase in activities of lesioned mice and iron diet restricted groups as compared to their respective controls [11].

This appears as promising animal model depicting restless legs syndrome helpful in developing drug treatment options as there was significant reduction in movements, aggression in diet deprived and A 11 lesioned mice which were put of D2 and D3 agonist thus validating concept of Dopamine pathways involvement in restless legs syndrome along with iron deficiency. It is also to be focused that major dopamine pathway may remain intact, inspite of which A 11 dopaminergic pathways are affected. In iron diet deprived followed by A11 lesioned mice group, aggression activity was observed interpreted as urge to move legs and increased anxiety levels of mice. Major concern is no feasibility of evaluating clinical features of restless legs syndrome like urge to move legs and other symptoms (not feasible in animal models) being mandatory criteria for diagnosis. During experiment D1 agonist in A11 lesioned mice actually led to increase in movements as compared to control group and is major limitation of study. Further increased activity of mice may be attributed to other aspects like attention deficit hyperactivity disorder or other movement disorders.

Barraud., *et al.* [12] conducted study on non-human primate (rhesus monkey n = 13) considering brain's anatomical similarity with humans as there have been challenges with translating rodent research models into humans. The group studied various aspects like a) distribution of dopamine receptors in spinal region (n = 4, *in situ* hybridization process was used), b) source of dopamine (n = 2, retrograde staining process), distribution including phenotypes of neurons of A11 region (n = 4 immuno-staining process, n = 3 high Performance Liquid Chromatography) and Parkinson's disease animal model based pathway study (n = 5, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced Parkinson's disease). While studying distribution of dopamine receptors in spinal region (n = 4) *in situ* hybridization process was used based on which dopamine receptors were identified at L2, L4 and L5 spinal level. Frontal brain sections of animals were taken as positive controls. It was observed that D2 and D3 receptors were most dense in spinal L2, L4 and L5 region with less concentration of D5 receptors. It is important to mention D1 receptors were not indentified in L2, L4 and L5 spinal regions of animals (Figure 10). This explains non-functioning of D1 agonist in A 11 lesioned mice as indicated by Le., *et al* [11].

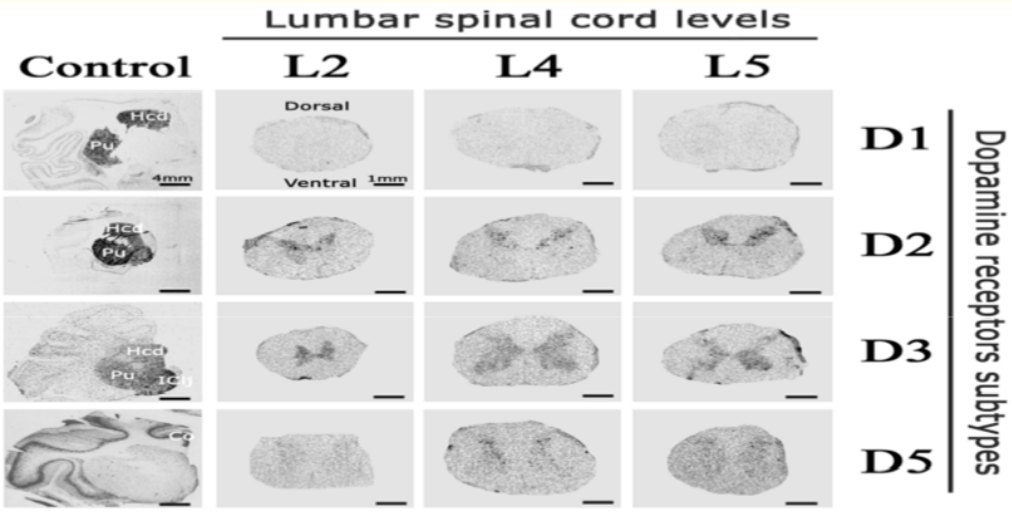

Figure 10: *Concentration of dopamine receptors at L2, L4 and L5 spinal level in study animals. Frontal brain sections of animals were taken as positive controls [12].*

Based on retrograde labelling technique, A11 dopaminergic neurons which communicate with spinal cord were identified and localized in study animals. Figure 11A and 11B represents hypothalamic area of animals where black dots represent location of A11 neurons.

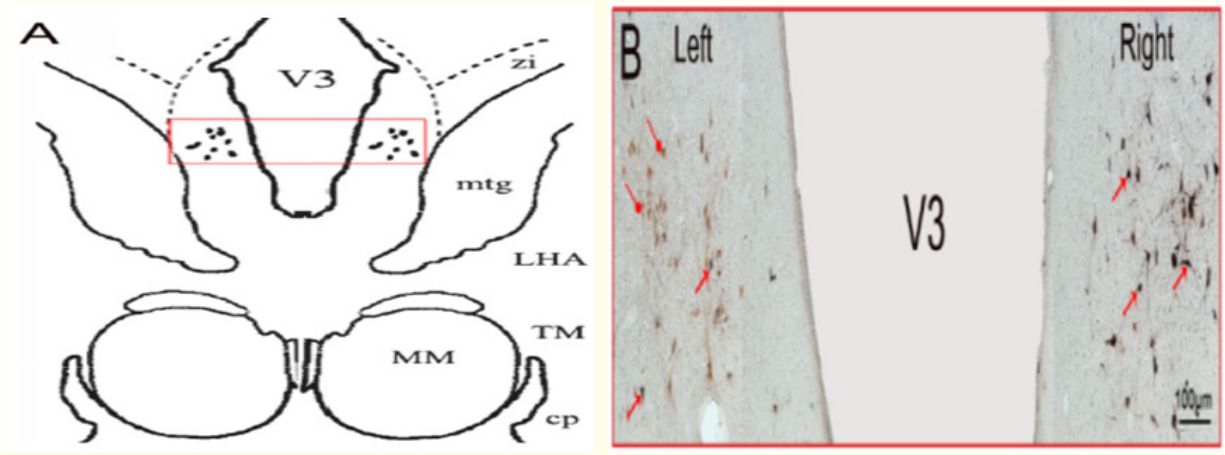

Figure 11: *A11 dopaminergic neurons localization in hypothalamic area of animals [12]. V3: Third Ventricle; LHA: Lateral Hypothalamic Area; TM: Tuberomammilary Nucleus; MM: Medial Mammillary Nucleus.*

In current study, key aspect was impact of methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced pathology in study animals. It was observed that concentration of A11 dopamine neurons was significantly reduced secondary to toxicity (n = 2 methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced toxicity group and n = 2 positive controls, p value < 0.0005) (Figure 12 and 13).

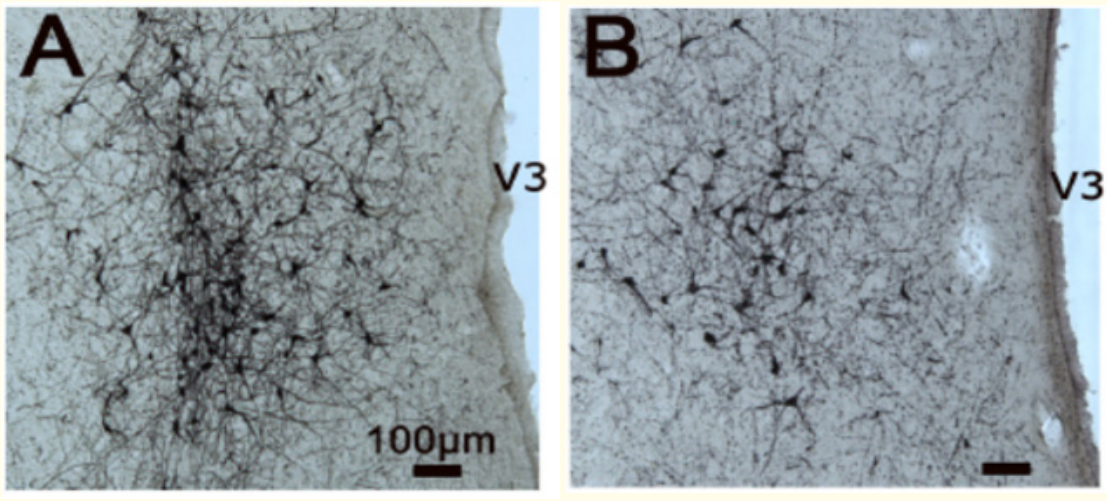

Figure 12: *Concentration of A11 dopamine neurons was significantly reduced secondary to methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity (n = 2 MPTP induced toxicity group B and n = 2 positive controls A, p value < 0.0005) [12].*

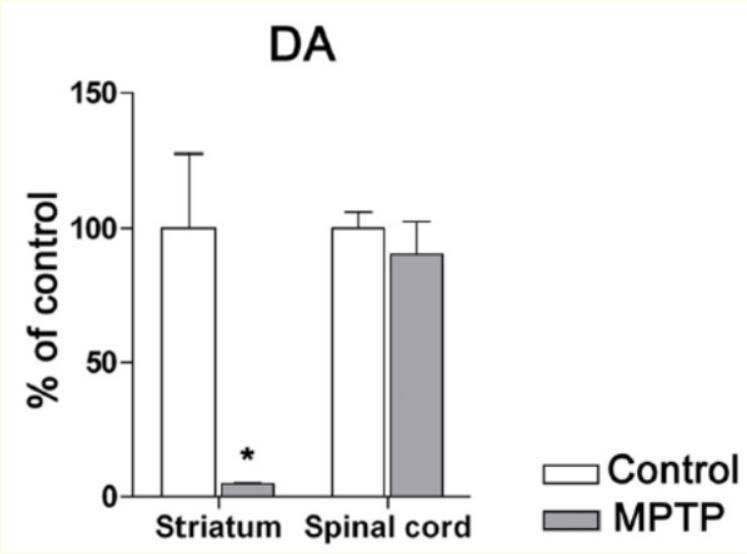

Figure 13: *No significant reduction in dopamine in Spinal Cord region post methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced toxicity as compared to controls [12].*

There was no significant reduction in DA in Spinal Cord region post A11 cell loss secondary to methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced toxicity as compared to controls. Contrarily, dopamine metabolites levels at spinal cord were decreased to a significant level indicating some compensatory mechanism decreasing dopamine degradation at spinal cord level.

Current study evidences location of A11 dopaminergic neurons, concentration of dopamine and its metabolites at various levels of brain and spinal cord. Hypothesis pertaining to diencephalospinal pathway links to earlier evidence and provides a strong base to further study concept, although number of animals studied were less. Hence current study provides significant rationale to hypothesis relating restless legs SYNDROME and A11 dopaminergic pathway [15-18].

Conclusion

In the current review multiple hypotheses pertaining to restless legs syndrome pathophysiology have been critically evaluated. Restless legs syndrome has been found to be associated with central nervous system disorders like Pontine infarction [3], gamma-aminobutyric acid neurotransmission involvement [4]. On the other hand, evidence suggested PNS pathophysiology theory as indicated by peripheral neuropathy studies. Another group focussed on opioid system and its relation with restless legs syndrome [8]. One of the meta-analysis provided evidence related to identification of 13 new genetic loci responsible for restless legs syndrome. Folate deficiency provided evidence of restless legs syndrome during pregnancy. One of the key important aspects evaluated was iron deficiency and its relation to restless legs syndrome [5]. Studies results suggested iron deficiency at blood level and cerebral tissues is linked to the pathophysiology of restless legs syndrome. Iron deficiency also had a role to play in the demyelination process of various neurons which in turn can lead to restless legs syndrome symptoms [6,7].

Strong evidence exists which differentiates the rodent and non-human primate/human neuroanatomy specially pertaining to certain types of dopamine receptors [11,12]. Overall, A11 dopaminergic neurons pathway links to earlier evidence and provides a strong base to further study the concept. In future more, robust animal models pertaining to restless legs syndrome and double-blind placebo controlled clinical trials need to be conducted to validate these theories.

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