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Connectome and molecular pharmacological dopaminergic differences in RLS: plastic changes and neuroadaptations that may contribute to augmentation

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Abstract

Restless Legs Syndrome (RLS) is primarily treated with levodopa and dopaminergics that target the inhibitory dopamine receptor subtypes D3 and D2. The initial success of this therapy led to the idea of a hypo-dopaminergic state as the mechanistic origin underlying RLS. However, multiple lines of evidence suggest that this simplified concept of a reduced dopamine function as the basis of RLS is incomplete. Moreover, long-term medication with the D2/D3 agonists leads to a reversal of the initial benefits of dopamine agonists and augmentation, which is a worsening of symptoms under therapy. The recent findings on the state of the dopamine system in RLS that support the notion that a dysfunction in the dopamine system may in fact give rise to a hyper-dopaminergic state is summaraized. Based on this data, the concept of a dynamic nature of the dopamine effects in a circadian context, is presented. The possible interactions of cell-adhesion molecules expressed by dopaminergic systems and their possible impact on RLS and augmentation are discussed. Genome-wide association studies (GWAS) indicate a significantly increased risk for RLS in populations with genomic variants of the cell adhesion molecule receptor type protein tyrosine phosphatase D (PTPRD), and PTPRD is abundantly expressed by dopamine neurons. PTPRD may play a role in the reconfiguration of neural circuits, including shaping the interplay of G protein-coupled receptor (GPCR) homomers and heteromers that mediate dopaminergic modulation. Recent animal model data support the concept that interactions between functionally-distinct dopamine receptor subtypes can re-shape behavioral outcomes and change with normal aging.

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Additionally, long-term activation of one dopamine receptor subtype can increase receptor expression of a different receptor subtype with opposing modulatory actions. Such dopamine receptor interactions at both spinal and supra-spinal levels appear to play important roles in RLS. In addition, these interactions can also extend to the adenosine A_{2A} receptor, which is also prominently expressed in the striatum. Interactions between A_{2A} and dopamine receptors and dopaminergic cell adhesion molecules, including PTPRD, may provide new pharmacological targets in treating RLS. In summary, new treatment options for RLS that include recovery from augmentation will have to take into account dynamic changes to the dopamine system that occur during the circadian cycle, plastic changes that can develop as a function of treatment or with aging, changes to the connectome based on alterations to cell adhesion molecules, and receptor interactions that may reach beyond the dopamine system itself.

Keywords

Restless legs syndrome; dopamine; receptors; PTPRD; heteromers; spinal

1. Introduction

Levodopa and dopamine $D_{2/3}$ receptor (D2/3R) agonists significantly improve the primary symptom of restless legs syndrome (RLS), the lower extremity akathisia [1, 2]. Dopamine (DA) antagonists, on the other hand, can trigger or worsen akathisia [3–5]. From these findings it has been deduced that the dopaminergic system is involved in the development of RLS/akathisia and that decreased DA function is at the heart of RLS [6–8]. The concept of decreased DA function in RLS has been the rationale for the proposed use of 6-OHDA-lesions and D3R knockouts to model RLS [9–12]. The problem is that the simplistic concept of “decreased DA function” cannot fully account for many other clinical features seen in RLS. How does “decreased DA function” account for the intensification of RLS/akathisia with chronic use of DA agonists or the distinct circadian presentation of the disease? As Parkinson’s disease exemplifies the ultimate state of “decreased DA function”, why do not all Parkinson’s patients develop RLS [13, 14]? Despite often-dramatic improvement in both RLS and periodic limb movement disorder (PLMD) with acute DA agonist treatments, polysomnographic data still show persisting abnormalities on other sleep measures [15]. Defining the underlying pathology of RLS as simply “decreased DA function” ignores obvious dynamic issues and clinical conundrums.

2. The state of the DAergic system in RLS

Some understanding of the dopaminergic system and its role in RLS can be derived from prior research. Cerebrospinal fluid (CSF) from individuals with RLS displays increased tetrahydrobiopterin (THB) and increased tyrosine hydroxylase (TH) activity [16]. CSF 3-ortho-methyldopa (3OMD) is increased in proportion to increased homovanillic acid (HVA), suggesting increased DA synthesis, release and turnover. CSF 3OMD and HVA levels are positively correlated with RLS severity and show diurnal variation with a nighttime nadir [17]. Postmortem putamen and substantia nigra samples from individuals with RLS display increased total and phosphorylated TH [18]. D2R densities are decreased in ways that

correlate with pre-morbid RLS severity [18]. Positron emission tomography (PET) imaging shows small but consistent decreases in D2R binding potential (BP) in the basal ganglia (BG) but no changes in estimates of Bmax or Kd [19]. The overall findings are interpreted as being due to increased synaptic DA. Autopsy and SPECT studies show no change in DA transporter (DAT), although PET studies do show decreased DAT in the basal ganglia [20]. The overall findings suggest decreased membrane-bound DAT without differences in total cellular DAT concentration. Thus, the simplified concept of "decreased dopamine function" as the basis of RLS fails in many ways to address both clinical and scientific data.

The data when taken together instead support the concept of a "hyper-dopaminergic" presynaptic state [21]: increased synthesis, release and decreased uptake of DA leading to increased synaptic DA. The findings also suggest a "hypo-dopaminergic" postsynaptic state with decreased D2/3R. Overall, the data suggests that a hyper-dopaminergic-presynaptic state in RLS is in balance with and/or in opposition to a hypo-dopaminergic-postsynaptic state [21]. Which components of the pre-post-synaptic interplay are primary, secondary or compensatory remains uncertain. Simplified models of the dopaminergic post- and pre-synaptic dynamics can be derived based on what is known from neuropharmacological modeling of neurotransmitter-receptor interactions [22]. In Model 1, if it is assumed that the primary system's problem starts with increased DA synthesis that results in increased synaptic DA with compensatory postsynaptic desensitization (i.e., decrease in the DA receptors) and feedback to increase DA uptake (increasing DAT). In Model 2, if it is assumed that the primary system's problem is decreased DAT, which results in increased synaptic DA, compensatory postsynaptic desensitization with decreased DA receptor numbers and feedback to decrease TH and DA synthesis. In Model 3, if it is assumed that the primary system's abnormality is a decrease in DA receptor signaling, which results in a feedback to pre-synaptic DAergic neurons, compensatory increase in TH activity and increased release of DA with decreased DAT and resulting increases in synaptic DA. The current data from studies on RLS best fit model 3. However, these simplified models are based on the assumption that only one element within this pre-post-synaptic dynamics is at fault for the imbalance in the dopaminergic system. That may not, however, be true. The iron-deficiency (ID) rodent model, which has demonstrated biological changes that closely mimic the changes seen in RLS [21], does implicate changes in dynamic relation between pre and synaptic function that are more complex than simple attribution of increased TH or decreased D2/3R as the primary insult [23]. Despite the uncertainty of what factors contribute to the dynamic change in the dopaminergic function in RLS, this is also an issue of systems dynamics: brain region, oscillatory state, feedback dynamics and other systems are likely to play primary roles in how dopaminergic system "functions" in RLS.

The concepts of altered pre- and post-synaptic dopaminergic activity, as discussed above, need to be framed within the greater dynamic of circadian mechanisms. The strikingly circadian nature of RLS symptom expression is a fairly unique clinical phenotype in neurology and is an integral element of the disease [24, 25]. Any model of RLS must include circadian dynamics. Circadian changes in human prolactin levels [26, 27], diurnal changes in CSF tetrahydrobiopterin in RLS patients [28], circadian presentation of DOPA-response dystonia [29] and the diurnal changes in PET-assessments of D2R affinity and density in the nucleus accumbens [19] all support the concept of a circadian dynamics in the human

dopaminergic system, even if they do not provide clear disease-specific differences in oscillation. This is further supported by studies in rodents [30–32] and monkeys [33]. If it is accepted that the akathisia is a dopamine-dependent clinical phenotype and thus a sensitive indicator of the "dopaminergic activity" then circadian oscillation in akathisia seen with RLS can be seen as a reflection of circadian oscillation in dopaminergic activity. Based on that assumption, the nadir of the dopaminergic -activity cycle would be at night or with sleep (i.e., when the RLS symptoms reaches their peak [24, 25]) and the peak of the dopaminergic -activity cycle would be in the in the morning (when RLS patients express relatively fewer symptoms [24, 25]). Although RLS appears to have normal circadian dynamics [34], it is proposed that intrinsic dopaminergic activity, though normal in its circadian periodicity, is relatively shifted downwards overall. This results in the intrinsic dopaminergic activity signal dropping below "critical levels" (that are needed to keep symptoms at bay) at the point of the nadir (at night) resulting in inadequate intrinsic dopaminergic activity and expression of RLS/akathisia. Intrinsic dopaminergic activity then rises rapidly to a peak in the early morning, corresponding to the "protected period" in RLS in which patients are most resistant to developing akathisia. This model of circadian dynamics of the intrinsic dopaminergic activity accounts for the circadian nature of RLS, for the short-term effectiveness of dopamine agonists and for the ineffectiveness and subsequent drug-induced augmentation with chronic agonist use [21], and at the same time suggests that the overall integrity of the dopaminergic system is intact.

3. Dopaminergic systems and cell adhesion molecules: how they may inform us about RLS and augmentation

Alterations in dopaminergic signaling has long been suspected to contribute to RLS. As there is no obvious disease-specific neuropathological findings as has been seen with Parkinson's disease or many other neurodegenerative disorders [35, 36], more subtle changes at the cellular level to explain the known iron-dopamine changes are likely to be involved. Differences in the microscopic cell-cell connectivities of dopaminergic neurons are potential candidates to play roles in the pathophysiology of RLS[37, 38]. To understand such RLS-associated differences, better elucidation of the molecular bases for connectivities of dopaminergic neurons, and of the possible differences in these molecular bases and connectivities in RLS are needed. From that biological base interventions that might normalize these RLS-associated molecular and connectivity differences could be developed.

Cell adhesion molecules may be potential candidates to play central roles in specifying the brain connectome [39]. Re-annotating the universe of likely cell adhesion molecules allowed us to divide them into groups that are more likely to provide anatomically-visible cell connections noted in electron micrographs and those that are more likely to transmit information between neurons and other cells of between neurons and signals attached to extracellular matrix [39]. It is proposed that "bar codes" of cell adhesion molecules make substantial contributions to the ways in which specific neuronal populations connect with development and even to the changes in these connections that come with experience and drug exposures.

RNAseq data (*generously shared by C Scherzer and colleagues*) indicate that more than 200 cell adhesion molecule genes are expressed by human dopamine neurons. One of these genes that is expressed at moderately abundant levels in these neurons encodes the cell adhesion molecule receptor type protein tyrosine phosphatase D (PTPRD). PTPRD is one of the genes that has been identified repeatedly as harboring variants that make "oligogenic" contributions to RLS in several genome wide association datasets [40–43]. PTPRD genomic variants can provide oligogenic, up to 1.3 – 1.8 x increases in risk for RLS.

PTPRD is abundantly expressed in mouse dopaminergic neurons as well (www.brain-map.org/search/index.html?query=Ptprd). This cell adhesion molecule is also expressed in targets of dopaminergic projections, including those in the cerebral cortex (larger, often pyramidal neurons in deeper cortical layers) and the striatum (large, apparently cholinergic interneurons). Since PTPRD can function as a homodimer, changes in dopaminergic circuits involving these neuronal types provides a plausible way of explaining how PTPRD differences could contribute to RLS-vulnerability-altering differences in dopaminergic connectome [44]. Though expression of PTPRD in other neurons and PTPRD's reported recognition of other binding partners enriches this picture [45–50], the focus will be on the dopaminergic circuits in which homodimeric recognition is likely to enhance connectivities in the descriptions below.

It has been recently reported that human postmortem cortex samples taken from individuals with some common PTPRD haplotypes express about 70% differences in levels of PTPRD expression compared to those taken from individuals with other PTPRD haplotypes [51]. These associations are significant for each of the single nucleotide polymorphisms (SNPs) that have been associated with RLS, and for a nearby SNP that provides the strongest nominal association. The observations suggest that PTPRD provides a significant part of the > 200-cell adhesion molecule "barcode" for connectivity of ventral midbrain dopamine neurons. They suggest that common PTPRD variants provide level-of-expression variation in PTPRD that, in turn, contributes to connectivity differences of dopaminergic and other brain systems that contribute to the pathophysiology of RLS. It is conceivable that these differences could also contribute to individual differences in vulnerability to augmentation when RLS is treated with dopaminergic agonists.

A study in mice with altered PTPRD expression has been reported [51]. Several observers provided blinded scoring of behaviors in the hour prior to and following onset of the normal sleep period. In mice with lifelong reductions in PTPRD expression, there was less sleep. Evidence for "pressure" to sleep came from longer sleep bout duration. When knockout mice entered behavioral sleep, they remained asleep for longer periods of time. These RLS-like phenotypes in knockout mice supports the idea that common individual differences in connections of neurons that express PTPRD contribute to the oligogenic influences of PTPRD variants on vulnerability to RLS.

By contrast, knockout mice displayed no differences from wildtype mice in periodic limb movement scores [51]. This was true for both the brief duration "twitch" and longer-duration movements that could be separate in the video observations from these mice. These

observations do not support the idea that limb movements provide the route through which sleep is disrupted in mice due to variation at the PTPRD oligogenic RLS-vulnerability locus.

Compounds that act to inhibit the phosphatase activities of recombinant phosphatase domains from human PTPRF have recently been identified [50]. Since PTPRF is a close member of the gene subfamily that contains PTPRD, a study synthesized analogs of the PTPRF ligands, prepared recombinant PTPRD protein tyrosine phosphatase domain protein, and tested the ability of these analogs to inhibit PTPRD phosphatase activity [52]. *In vitro*, these illudalic acid analogs provided $< 10^{-5}$ M potency in inhibiting recombinant human PTPRD phosphatase activity in our initial experiments. These encouraging results are only the first steps in the long program of work required to produce a drug useful in humans. Nevertheless, drugs that act at PTPRD, including those that derive from recently-elucidated illudalic acid lead compounds, may be good candidates for *in vivo* testing to seek toxicities and even influences on sleep parameters in wildtype and PTPRD knockout mice. Conceivably, compounds that could selectively modulate PTPRD activities could provide selective alterations in dopaminergic connectivities in ways that would ameliorate symptoms of RLS. These activities might be more important for individuals with the PTPRD risk alleles, but might also be useful for those with RLS caused by other genetic and environmental sources.

These observations mesh with those of other portions of this paper in several ways. Allen brain atlas *in situ* hybridization data supports possible expression of PTPRD in A11 regions that contain dopamine neurons that project to the spinal cord. In the spinal cord, abundant PTPRD expression for anterior horn presumed motor neurons is accompanied by expression in other interesting cell types as well. PTPRD ligands might thus also impact dopamine projections to the spinal circuitry. Other cell adhesion molecules expressed by dopaminergic neurons include several that are likely to be concentrated in lipid raft zones *via* glycophosphatidyl inositol (GPI) anchors [39]. These lipid raft zones are enriched in dopamine, adenosine and other G-protein coupled receptors [53, 54]. The geometries of homomeric and heteromeric cell adhesion molecule recognition of their ligands on other neurons allow anchoring of such rafts in areas in which pre- and post-synaptic neurons are closely opposed to each other [39]. Thus, the monomeric, dimeric, and heteromeric GPCR receptor complements described below could be exposed to basal and transient changes in dopamine concentrations different from those to which they might have been exposed without such anchoring in zones of close apposition of these cellular membranes.

Augmentation could conceivably be due to circuitry changes that were effected, at least in part, due to altered expression of dopaminergic cell adhesion molecules that might even include PTPRD. Better understanding of cell adhesion molecules, including PTPRD, may provide a better understanding of the circuitry and specific connectome differences involved in biological underpinning of RLS and possibly augmentation that comes from chronic treatment with dopamine agonists.

4. The potential role of D1-D3 receptor interaction in RLS

Although the DAergic drugs used in RLS are D2/3R agonists, D3R effects, more so than D2R effects, appear to be most relevant to treatment efficacy [55]. There are several RLS models based on the concept of altered D3R function [12]. There are limited data to also support the potential role of D1R, at least in the development of akathisia [3]. It is proposed that D1 and D3 receptor ligand sensitivity, secondary signaling or receptor interactions may play a role in either the development of symptoms or the development of augmentation.

D1-like receptors (D1R and D5R) activate G protein pathways via excitatory G*s*-coupled second messenger pathways that in turn increase cAMP levels and cellular excitability, while D2-like receptors (D2R, D3R, and D4R) activate inhibitory Gi-coupled pathways, which reduce the activation of cAMP-mediated pathways and decrease cellular excitability [56, 57]. Both D1-like and D2-like receptors can be found on the same neurons [58], and DA can up- or downregulate cellular and network functions in a dose-dependent manner [57, 59, 60]. Moreover, modeling studies of mammalian DA neurons suggest that tonic versus burst firing can result in differences in the relative occupancies of the different receptor subtypes [61, 62]. D3R and D1R can display functional interactions that are based on different hetero-dimer [63–65] or hetero-tetramer configurations [66]. In the hetero-tetrameric model, D1R and D3R co-activation leads to both antagonistic and synergistic interactions at the level of adenylyl-cyclase and MAPK activation, respectively [66]. Experimental evidence supports the idea that D1R-D3R antagonistic interactions play an important role at the spinal cord level [67], while synergistic interactions might be more involved at the striatal level [63]. For example, activation of D1R tends to increase the excitability or the performance of neural networks that underlie or control fictive locomotion in different animal models [59, 67]. In contrast, activation of the D3R pathway reduces overall motor excitability [68]. A dysfunction of the D3R system is also associated with a significant decrease in thermal pain withdrawal behavior [69]. Such a heat-dependent hyperalgesia suggests that D3R mediate the excitation levels in the underlying spinal sensory circuitries that mediate noxious inputs received from C-fibers. Importantly, a dysfunction of the D3R system in D3R knockout mice (D3RKO) is associated with an increase in D1R protein expression levels in the spinal cord [70]. As D1R and D3R often co-localize or form heterodimers and oppositely regulate cAMP/PKA-mediated second messenger pathways, the authors postulated that, in D3RKO, the dysfunction of the D3R receptor prevents the D3R-mediated block of AC, and leaves D1R actions unopposed. Under such circumstances, cAMP pathways might be continuously upregulated, and additional application of cAMP nucleotides might fail to further increase cellular excitability. These data suggest that a failing D3R system, as possibly also present in augmented RLS patients that have undergone long-term D3R treatment, might give rise to alterations in the D1R system, which could then account for the reduced effect in the original treatment and the augmented state.

The prevalence of RLS increases with age [71] suggesting the possibility of an age-dependent mechanism in the development of RLS. Normal aging is associated with neuronal loss in the striatum [72], a decrease in overall DA levels [73], and a decrease in D2-like receptor expression (D2R, D3R, and D4R) [74, 75]. As the D2-like receptors have, overall, a higher affinity to DA than D1-like receptors [76], and as D2-like receptors mediate overall

inhibitory modulatory effects, these data suggest that aging may be associated with a gradual disinhibition of the DA system. In this scenario, aging RLS patients would likely require higher doses of the D3R agonist than younger patients, to compensate for the parallel reduction in DA-mediated inhibition in their systems. Alternatively, and due to the receptor interactions between D3R and D1R, a relative increase in D1R levels would lead to a similar result, i.e. a reduced effectiveness of the D3R agonist. In support of this scenario, one study showed that normal aging in rats is associated with an opposing aging-related shift of excitatory dopamine D1R and inhibitory D3R protein expression in both striatum and spinal cord [77]. Specifically, using Western blot analyses, D1R expression levels were increased in both the striatum and spinal cord 3 to 5 – fold from 2 months to 2 years of age, and immunohistochemistry suggested that the increase of D1R expression in the spinal cord was more predominant in the ventral (motor) areas than in the dorsal (sensory) areas. In contrast to the increase in D1R expression, D3R expression levels did not differ significantly over the lifespan of the animals. These data suggest that the overall D1R-D3R ratio shifts with normal aging towards a more excitatory DA receptor phenotype. At the very least these data suggest that exploring age-dependent models of D1R and D3R sensitivity or interaction may provide important insight relevant to understanding RLS pathology.

As long-term treatment with D3R agonists alone can lead to an increase of D1R expression and reverse analgesic effects into hyperalgesic responses (Lallemand et al., personal communication), it is tempting to speculate that the development of augmentation in RLS with chronic use of D2-3R agonists may be in part due to the a changing D3R versus D1R environment.

5. Receptor heteromerization and relevance to RLS

It is becoming generally accepted that G protein-coupled receptors (GPCR) are not simply single functional units. GPCR form functional complexes with other receptors referred to as receptor oligomers [78, 79]. A receptor oligomer is defined as a macromolecular complex composed of at least two (functional) receptor units with biochemical properties that are demonstrably different from those of its individual components [78]. The focus of this presentation will be on GPCR heteromers, which is when two or more molecularly different receptors form a functional unit (e.g., D1R-D3R complex or A2AR-D2R complex). The physical binding of two or more receptors into a “functional” unit can significantly influence the allosteric nature of the ligands that bind to either receptor, that is the affinity of the ligand (or endogenous neurotransmitter) and its downstream functional signal (intrinsic efficacy). A second element of the oligomeric unit is the effects that a ligand (or endogenous neurotransmitter) binding to one receptor in the complex will have on the affinity or efficacy of a ligand (or endogenous neurotransmitter) binding to the other receptor in the unit [80]. Finally, formation of an oligomeric complex may influence its turnover-degradation, which may also be further influenced by ligand binding to one or the other receptors in the complex. The studies into the interaction of the well-established adenosine A_{2A} receptor (A2AR)-dopamine D_2 receptor (D2R) heteromer unit provide good examples of this complex pharmacology [81]. The physical binding of D2R to A2AR to form a heteromer resulted in the decrease of the ability of a specific A2AR antagonist to bind to the A2AR-D2R heteromer [79, 81]. Within the A2AR-D2R heteromer, when ligands bind to the A2AR,

it dramatically affects the affinity of D2R ligands. This provided the basis for the use of A2AR antagonists as an adjuvant to levodopa treatment in Parkinson's disease [82]. Screening with various *in vitro* and *in vivo* techniques led to the finding of very different qualitative properties of several selective A2AR antagonists [83]. The most striking finding was a change in the binding properties of SCH 442416 for A2AR when forming heteromers with D2R, compared to when not forming heteromers or forming heteromers with adenosine A_1 receptor (A1R) [83]. A1R-A2AR heteromers are localized in striatal glutamatergic terminals [84] and in preclinical studies SCH 442416 has provided a tool to selectively target striatal presynaptic versus postsynaptic A2AR [85, 86].

Both supra-spinal and spinal mechanisms have been invoked as being involved in the pathophysiology of RLS [87]. The supra-spinal mechanisms favor a predominant subcortical, striatal impairment of sensorimotor integration [88, 89]. In fact, the striatum is the brain area with the highest dopamine innervation and the highest density of dopamine receptors in the brain, the main point of interaction of dopamine within the cortical-striatal-thalamic-cortical circuits. GABAergic medium spiny neurons (MSN) constitute the most abundant striatal neuronal population [90]. There are two subtypes of MSN, which define two striatal efferent pathways connecting the striatum with the output structures of the basal ganglia. A2AR-D2R heteromers are selectively localized in the indirect MSN, which gives rise to the indirect pathway [90]. The direct MSN constitutes the direct efferent pathway and expresses D1R and D3R, which can potentially form D1R-D3R heteromers [63, 65]. D3R has a lower striatal expression than D1R, but it upregulates in animal models of L-dopa-induced dyskinesia and psychostimulants abuse [91, 92]. Significantly, striatal D3R have also been reported to upregulate in cocaine- and methamphetamine-dependent subjects [86, 93] and D1R-D3R heteromers have been suggested to play a main pathogenetic role in these disorders [94]. Thus, a previous study indicated a synergistic locomotor activity induced by D1R and D3R agonists in reserpinized mice [65]. In a recent study, evidence has been provided for a functional selectivity of allosteric modulations within the D1R-D3R heteromer, a negative cross-talk at the adenylyl-cyclase level and a positive cross-talk of both receptors on MAPK signaling, which can be involved with the reported behavioral synergism of D1R and D3R agonists [95]. Significantly, a recent study found a potentiation of D1R by D3R activation at the MAPK signaling in an animal model of L-dopa-induced dyskinesia [92]. Contrary to what was hypothesized in relation to the spinal mechanisms, a D3R-mediated potentiation of D1R-mediated MAPK signaling at the striatal level could be involved in augmentation, but in the frame of the D1R-D3R heteromer.

In addition to targeting the direct MSN, preliminary results obtained in the rodent iron-deficient model of RLS suggest an increased in presynaptic cortico-striatal glutamatergic neurotransmission (Yepes et al., in preparation), which is associated with upregulation of A2AR [66]. Therefore, striatal presynaptic A2AR, i.e. A1R-A2AR heteromers, represent a possible new target for RLS. In addition of A1R-A2AR heteromers, D2R-D4R heteromers are postulated to exert an important control of striatal glutamate release [96] and therefore are also putative targets for RLS. It is in fact possible that the D2-like receptor agonists utilized clinically in RLS (ropinirole, pramipexole, rotigotine and even levodopa) exert part of their therapeutic effect by acting on D2R-D4R heteromers. In summary, it is proposed

that striatal adenosine and dopamine receptor heteromers can constitute new targets for the treatment of RLS.

6. Summary, Keys Points and Future Directions

1. Models of dopamine-dependent behavior that go beyond the standard knockout and lesion models that alter the dopaminergic pathway in relatively static ways are needed. Improved models should reflect true pre-/post-synaptic dynamic changes in intact and normally-functioning dopaminergic system interactions as well as changes in these dynamic systems that reflect hyperdopaminergic presynaptic condition. Models developed for ADHD may provide guidance. The iron deficiency (ID) rodent model is one model that does fill the criteria and data derived from that model implicate presynaptic dynamics (increased tyrosine hydroxylase synthesis and decreased DAT) as a primary biological change.
2. Any model developed on the basis of pre-/post-synaptic dopaminergic system interactions also needs to include factors (iron homeostasis, glutamatergic, adenosinergic, cellular metabolism, cell adhesion molecule mechanisms) which govern or interact with the dopaminergic system. As an example, the GLT-1 is the primary glutamate uptake transporter for astrocytes. It controls synaptic glutamate and thus controls glutamate effects on other systems like DAergic. Genetic expression negatively correlates with has VMB iron in mice. ID also negatively effects GLP-1 expression. Thus, there is a link between iron homeostasis, glutamate and DAergic system that could be relevant to RLS biology.
3. Finally any modeling system needs to demonstrate variability of outcomes that follows circadian dynamics.
4. Chronic enhancement of the dopaminergic system causes further progression of the disease or augmentation. Augmentation is an important clue as to the dopaminergic dynamics in RLS and should not be ignored as simply just a side effect of the drug.
5. DA can initiate highly diverse responses in the affected neural circuits that are 1) dependent on the levels of the neurotransmitter released, 2) dependent on the receptor subtype activated, 3) dependent on the time exposure of the duration of a treatment, or 4) dependent on age. Understanding these dynamic interactions and deciphering their molecular mechanisms in more detail will be crucial in solving the puzzle of augmentation in the DA treatment of RLS.
6. Receptor oligomerization is becoming most accepted in the field of GPCR physiology and pharmacology. Particularly important is the possible role of receptor heteromerization in the pathophysiology of neuropsychiatric disorders, which changes their normal expression or function. In particular dopamine and adenosine receptor heteromers that modulate cortico-striatal glutamatergic transmission constitute possible new therapeutic targets for RLS.

7. The function of the known RLS risk genes within the context of their normal physiologic or biological role within the cell (as presented above with PTPDR) need to be studied. How these genes might interact with each other and thus develop a disease-specific, gene-based "connectome" would be the next level of exploration. Finally, the effects of known risk factors (iron deficiency) or biological conditions (i.e., circadian dynamics) have on these genes, their cell function/expression and gene-gene dynamics needs to be studies.

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Highlights

- Long-term treatment of RLS dopamine D2/D3 receptor agonists leads to augmentation
- Augmentation may be a consequence of a hyper-dopaminergic state
- Protein tyrosine phosphatase D (PTPRD) may play a role in the reconfiguration of neural circuits
- Alterations in direct and indirect interactions between D1 and D3 receptors might be involved
- New treatment options for RLS may reach beyond the dopamine system itself