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Consensus Guidelines on Rodent Models of Restless Legs Syndrome

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Abstract

Restless legs syndrome (RLS) is a chronic sensorimotor disorder diagnosed by clinical symptoms. It is challenging to translate the diagnostic self-reported features of RLS to animals. To help researchers design their experiments, a task force was convened to develop consensus guidelines for experimental readouts in RLS animal models. The RLS clinical diagnostic criteria were used as a starting point. After soliciting additional important clinical features of RLS, a consensus set of methods and outcome measures intent on capturing these features—in the absence of a face-to-face interview—was generated and subsequently prioritized by the task force. These were, in turn, translated into corresponding methods and outcome measures for research on laboratory rats and mice and used to generate the final recommendations. The task force recommended activity monitoring and polysomnography as principal tools in assessing RLS-like behavior in rodents. Data derived from these methods were determined to be the preferred surrogate measures for the urge to move, the principal defining feature of RLS. The same tools may be used to objectively demonstrate sleep-state features highly associated with RLS, such as sleep disturbance and number and periodicity of limb movements. Pharmacological challenges and dietary or other manipulations that affect iron availability are desirable to aggravate or improve RLS-like behavior and lend greater confidence that the animal model being proffered replicates key clinical features

of RLS. These guidelines provide the first consensus experimental framework for researchers to use when developing new rodent models of RLS.

Keywords

guidelines; animal models; RLS; Willis-Ekbom disease

Restless legs syndrome (RLS) is a common sensorimotor disorder clinically defined by an urge to move the legs, usually associated with unpleasant sensations that are induced or exacerbated by inactivity and relieved by movement, and that emerge or worsen in the evening or at night. RLS has a considerable negative impact on sleep and is associated with significant morbidity and substantial economic burden.[1] RLS is diagnosed through clinical interviews during which subjective symptoms are evaluated. Periodic leg movements of sleep (PLMS)[2] are one of the supportive diagnostic features of RLS, and although their specificity for RLS is low, they represent a unique objective measure for RLS.[3]

RLS symptoms respond well to low doses of dopamine agonists (DA), $\alpha_2\delta$-ligands, or opiates.[4] Still, a subset of patients remain refractory to treatment or experience serious adverse effects or loss of efficacy over time. In particular, long-term treatment with DA is frequently associated with augmentation, which is a severe drug-related paradoxical worsening of symptoms. Augmentation is the main reason for DA withdrawal and the most difficult challenge in clinical practice. Therefore, new treatments and prevention methods are urgently needed. A systematic preclinical research program would have the potential for identifying novel druggable targets and accelerating the transition of novel drug candidates to clinical testing. Animal models could be key to achieving a mechanistic understanding of RLS and facilitating efficient platforms for evaluating new therapeutics.

In this light, it is encouraging that several translational models of RLS have emerged. The equivalence of RLS behavioral features between animal models and humans is, however, difficult to ascertain since the core RLS feature is a subjective sensory experience. Furthermore, there is a great potential for methodological variability in studies of animal models of RLS, which may hinder the comparison of study results and the assessment of the external validity of the models. These factors highlight the urgent need for a reference guideline for RLS-like behaviors in rodents. Human behaviors and phenotypes that are proxies for RLS need to be agreed upon, which, in turn, can generate comparable and objective behavioral analogs in potential RLS animal models. This work provides an expert consensus on key aspects of translational research in RLS needed to develop a standardized preclinical framework to model this human disease in animals.

Methods: Process for Developing Guidelines

In July 2018, the International RLS Study Group (IRLSSG) approved the formation of a task force to develop consensus guidelines on RLS animal models. The task force was composed of six researchers experienced in working on animal models of RLS/PLM (hereafter “basic scientists”; A.V.S., A.S., J.M.S., S.F., Y.L., and S.C.) and six clinicians (hereafter “clinicians”; M.M., R.P.A., W.O., D.R., D.L.P., and D.G.B.) experienced in RLS diagnosis

and treatment as well as in translational research. Two supervisors (J.W.W. and I.G.) reviewed the methods and outcome measures produced.

The Delphi method[5] was modified to suit the specific needs of this task force. The Delphi method is a structured communication technique based on several rounds of controlled feedback that enables the opinion of experts to converge so as to reach a consensus on a specific theme. In this guided process, each expert answers specific questions from a moderator, in a written and anonymous form, thereby avoiding pitfalls and biases of direct confrontations. This approach enabled group interaction and encouraged the exploration of ideas in preparation for the final face-to-face meeting, while at the same time preventing any single task force member from exerting undue influence over the proceedings.

Guideline development consisted of two phases, each comprising multiple rounds through 12 telephone conferences, email correspondence, and two face-to-face plenary meetings. All participants approved the aims and the general methodology of the task force. Given the areas of expertise of the task force and the work published in the field so far, it was decided to restrict the guidelines to rodent models. An agreement on definitions of terms used appears in Table 1.

Phase 1: Generation of Key Clinical Features

The clinicians generated a list of clinical features (Table 2) considered relevant for the diagnosis of RLS.[6] This step served to expand the breadth of diagnostic criteria, leveraging the substantial experience of the clinicians in the task force. The clinicians then voted on whether to include or exclude each of these new proposed clinical features: items receiving <4 out of 6 positive votes were excluded.

In a second step, each clinician anonymously proposed methods and outcome measures for assessing each of the clinical features in humans in the absence of a clinical interview and without restrictions (technological or financial).

Phase 2: Basic Science Methodology

All the RLS clinical features and proposed assessments were provided to the basic scientists for review. Any questions on these proposals were answered in writing by the clinicians.

The basic scientists then proposed methods and outcome measures that (1) could be applied in rodents and (2) closely corresponded to the proposed human clinical methods and outcome measures.

Phase 3: Prioritization

Clinical Features—All retained clinical features, including the supportive diagnostic criteria, but not the essential criteria, were subsequently categorized anonymously by each of the clinicians. In this classification, “A” was defined as “the presence or absence of this feature reinforces or weakens the diagnosis of cases which, despite fulfilling the essential criteria, remain uncertain”, while “B” was defined as “the presence or absence of this feature needs to be considered in collecting the medical history but taken alone does not change the

diagnostic decision of RLS". To receive a final "A" grade, a clinical feature had to receive at least three "A" votes from the six clinicians.

Clinical Methods and Outcome Measures—Each proposed clinical method and outcome measure was scored by the clinicians according to how valuable it was perceived to be in assessing the different clinical features (from 0: not reliable to 5: highly reliable). Any item receiving more than three 0 scores was excluded; the retained methods for each of the clinical features were ranked based on their total score. Finally, each method and outcome measure was categorized as "A" if it obtained a mean score >2.5 or as "B" if ≤2.5.

Methods and Outcome Measures for Animal Models—Each basic scientist graded each animal model method and outcome measure according to how well it was perceived to translate the corresponding clinical method or outcome measure on a scale from 0 (rejected) to 5 (very well). Any items receiving three or more 0 scores were excluded from the final list. Finally, each retained item was graded and categorized as "A" if it obtained a mean score >2.5 or as "B" if ≤2.5.

Phase 4: Plenary Meeting

In the final face-to-face meeting, both clinicians and basic scientists reviewed the process undertaken, summarized the results, and established endpoints (definition in Table 1) for the clinical and animal model outcome measures. All clinical features were retained irrespective of grading in the final document to produce guidelines based on the entirety of the clinical picture of RLS. To increase the robustness of recommendations, the plenary meeting discussed only animal models and clinical methods and outcome measures graded as "A".

After approval of the written report by all task force members, the recommendations were forwarded to the IRLSSG Executive Committee for review and endorsement. Figure S1 provides a flowchart of the whole process employed to generate these consensus guidelines.

Results

The results of the clinical feature grading are reported in Table S1. The clinical features of RLS and the corresponding recommended animal model methods and outcome measures were grouped into three main categories: essential diagnostic criteria (Table 2), PLMs and sleep (Table 3), and responses to pharmacological interventions or iron deficiency (Table 4). The corresponding clinical measurements proposed by the task force are mentioned in the following paragraphs. Additional supportive clinical features related to RLS history, sex, and age were also discussed and are reported at the end of this section. A concise summary of the clinical features for the translation of RLS-like phenotypes is provided in Table S2.

The first category of clinical features includes the five essential criteria for RLS diagnosis[6] and additional criteria concerning the temporal and spatial localization of RLS symptoms (Table 2).

Assessing the Urge to Move and Relief with Movement

An urge to move the legs, usually accompanied by unpleasant/uncomfortable sensations, is the core feature of RLS. This symptom is induced or exacerbated by rest and inactivity, such as the relaxed wakefulness preceding sleep onset, and is relieved—at least transiently—by movement. This symptom is most prominent in the legs and occurs most frequently during the evening and at night. In the absence of direct verbal communication, such as a clinical interview, it is challenging for the observer to identify and quantify the intensity of the patient's sensory experience. Therefore, the clinicians focused on surrogate measures, particularly activity, and the behavioral expression of discomfort.

The diagnostic criteria[7] derived in 2003 for young children and the cognitively impaired are prime examples of how an RLS diagnosis can be derived when communication is not fully possible. Herein, the diagnosis relies upon observing motor activity, which was considered an implicit manifestation of the urge to move, employing video analyses of behavior and movements, video polysomnography (vPSG), actigraphy (even in more than one limb), or any suitably validated activity monitor. For animal models, the urge to move can be similarly inferred from the observation of increased movement during a period when the animal would otherwise be expected to show decreased spontaneous movement or express behavioral quiescence.

In clinical settings, the urge to move in RLS patients when at rest may be objectively quantified by the suggested immobilization test (SIT).[8] However, the SIT relies on instructions communicated to the patient, which challenges direct translation of this method to animal research. Forced immobilization or Pavlovian conditioning of the animals to stay still were discussed as alternative approaches that might overcome this hurdle. These might help discriminate the "urge" or "drive" to move when resting from generalized activity increase, but the behavioral task demands may be alerting and alter the underlying condition. The task force recommended caution in the application of these methods to RLS animal models until further validation.

Activity Monitoring

The possible methods used for activity monitoring in animals include, but are not limited to, video tracking,[9] piezoelectric floor sensors,[10] infrared or laser beam grids,[11] implanted accelerometers,[12] activity telemeters,[13] voluntary wheel running,[14] and neck or limb electromyography (EMG) recordings.[15] Piezoelectric floor sensors, laser grids, accelerometers, and telemeters record all types of movement but are limited in further classifying movement context. EMG recordings measure muscle activation, which may not always translate to movement. Video tracking allows discrimination between locomotor and non-locomotor leg movements but may not be sensitive enough to detect low-amplitude phasic myoclonic bursts. The method of choice should be dictated by the individual experimental design.

Increased total activity, increased distance traveled, and decreased time at rest were initially proposed by the task force as surrogate measures for the urge to move. The use of rest and activity indices is limited by the lack of normative data for each method proposed. Rest

episodes, operationally defined as periods of very low or no activity, may include short periods of sleep. Waking activity may account for only 50% of the variance of the duration of rest episodes.[16] Respecting these confounders, the task force refrained from making more specific recommendations on the definitions of rest and activity episodes.

Activity monitoring with both forelimb and hindlimb EMG recordings is a further recommended method. However, while bilateral hindlimb EMG recordings have been shown to be feasible in rats and mice,[15] surgical approaches for the additional simultaneous forelimb EMG recording would need to be developed and standardized.

Assessment of Sensory Limb Discomfort

Although many RLS patients report symptoms in their arms,[17] leg discomfort almost always precedes arm symptoms. Therefore, the clinicians suggested two additional specifiers: behavioral manifestations consistent with discomfort being experienced in the legs and their distal extremities, and distal predominance of symptoms. The suggested clinical methods included video analysis of behavior, with a focus on touching or rubbing of different body parts and potentially in combination with SIT, and four-limb actigraphy, with a focus on differences between arm and leg activity. The task force considered it preferable to focus on the observation of rodent hindlimbs, which anatomically correspond more closely to human legs, although it recognized that further research on the functional correspondence of human arms and legs to rodent forelimbs and hindlimbs is needed, particularly during sleep.

The methods proposed to assess sensory discomfort in animals include video recordings, activity monitoring, and vPSG. Ideally, video recordings for this specific application would be performed with multiple high-resolution cameras, as rodent hindlimb movements may be tiny and the animal body may prevent hindlimb imaging from a single camera. Video recordings should be performed in the animal's home cage and be used to assess the number and characteristics of hindlimb activity events that could reflect either a response to sensory discomfort or the activity to relieve symptoms, as opposed to increased exploratory activity in a novel environment. Outcome measures to assess sensory discomfort are detailed in Table 2. However, quantification of video-recording results may be problematic, and researchers should consider standardizing and validating semi-quantitative scales.

Assessment of Evening/Night Preponderance of Symptoms

The clinicians proposed two specifiers relating to the timing of the symptoms: (1) the urge to move the legs is more likely or more intense in the latter part of the main wake period (evening) including the transition from wake to the main sleep period, and potentially extending to wakefulness bouts throughout the main sleep period, and (2) a circadian pattern of signs and symptoms. The suggested clinical methods included 24-hours video analysis of movements or actigraphy coupled with measurement of a circadian marker such as salivary melatonin concentration or core body temperature. On this basis, the task force recommended that the translation to rodent models should include the occurrence of a peak of behavioral signs in the last part of the dark period and/or the first part of the light period, coinciding with the transition from the main period of activity into the main period of sleep. This may extend to prolonged bouts of wakefulness throughout the light (inactive) period.

To have a complete circadian description of motor activity, the duration of observation should be at least 24 hours and preferably several days for internal validation, regardless of the method used. As the effects of ambient light on behavior may differ between rodents and humans, the changes in RLS-like rodent behaviors with changes in light may not fully translate to those in humans. Only in case of specific research questions that require the researchers to discriminate between circadian versus light-dependent rhythms and to properly characterize the circadian rhythms in the absence of the masking effect of light, the task force recommended including prolonged experiments in constant darkness, according to the standards of chronobiological practice.[18] Moreover, given that access to food is a potent Zeitgeber that may mask circadian influences in small animals, it is recommended that food be provided ad libitum. The social environment of the animals (single or group-housing) may affect circadian rhythms. The task force recommended single-housing during monitoring until more data become available on the impact of social cues on RLS-like behavior in rodents.

Assessing Possible Mimics of RLS

The task force recommended that the fifth essential RLS diagnostic criterion, the exclusion of mimics, also be respected in RLS animal models by excluding myopathy, neuropathy, and severe arthritis, ideally by way of formal pathological assessment. This recommendation must be tempered by the extra cost and effort associated with such an assessment. In particular, this assessment may be particularly relevant for models with novel genetic mutations or for models of other diseases that can potentially include these mimic conditions in their clinical spectrum, whereas it may be levied when experimental factors potentially contributing to RLS mimics are carefully controlled for. In uncertain cases, the provision that potential mimics are not solely accountable for the RLS-like phenotype could be translated by demonstrating at least partial behavioral phenotype rescue with pharmacological challenges (see later).

Clinical Features Related to Periodic Limb Movements and Sleep

These clinical features and their recommended translation to animal models are detailed in Table 3.

Periodic Leg Movements

International standard guidelines for PLMS recording and scoring recommend EMG recordings of both tibialis anterior muscles.[2] PLMS are the most common leg movement category during sleep in adult patients with RLS, and their occurrence at rates greater than expected for age or medical/medication status is a supportive criterion for RLS diagnosis.[6] PLM may also occur during wakefulness (PLMW) in patients with RLS.

Bilateral tibialis anterior EMG recordings are feasible in rats and mice.[15] The task force agreed that thresholds for animal models should be data-driven, as there would be no basis to assume a priori that the thresholds that define human PLMS are duplicated in other animals. Nevertheless, the principles informing human standards should represent a starting point for the discovery and definition of new standards for rodents. The analysis of tibialis anterior EMG bursts in healthy rats and mice during non-rapid eye movement (REM) sleep

showed that the majority would not be considered periodic since they occurred in short runs (<4) separated by inter-burst intervals <10 seconds, matching the pattern of short-interval leg movements during sleep (SILMS) observed in healthy humans without PLMS.[15] The evidence for a PLMS-like peak in inter-burst interval distribution at values >10 seconds was recently reported for the first time in rats subjected to an iron-deficient diet previously shown to produce brain iron deficiency.[19] In this study, the peak of the inter-burst interval distribution was between 10 and 20 seconds. The task force emphasized that more studies are needed to determine the real translational value of these findings and to extend the results to mice. Another possibility that cannot be discounted at present is that rodent models of RLS are characterized by an increased occurrence of non-periodic tibialis anterior EMG bursts during sleep. This would be in line with the data that PLMS make up less than 20% of total leg movements during sleep in children and adolescents with RLS,[20] and that adult patients with RLS have significantly greater frequency not only of PLMS, but also of non-periodic leg movements during sleep, including SILMS, compared with healthy controls.[21]

Consequently, the task force recommended that the occurrence of tibialis anterior EMG bursts be reported as a function of the distribution of inter-burst interval and, ideally, also of the number of bursts per run, to allow clear comparison with data in other animal models and humans. In the present stage of knowledge, not only PLMS but any increase in tibialis anterior EMG burst activity during sleep may be considered RLS-like behavior in rodent models.

Sleep Disturbance

Although sleep disturbance is not an essential diagnostic clinical feature of RLS, sleep in moderate to severe RLS is often interrupted or delayed in association with the urge to move. Sleep disturbance is included as a supporting feature in RLS diagnostic guidelines[6] and correlates with disease severity.[22] It is plausible that PLMS contribute to sleep fragmentation independently of sensory disturbances, although this has not been comprehensively assessed. Clinicians identified three clinical sleep parameters as valuable in RLS research: long sleep latency, reduced total sleep time, and more frequent sleep state transitions/fragmented sleep. The reported changes in the percentage of total sleep time spent in REM sleep or in slow-wave sleep in patients with RLS were not considered sufficiently robust to be recommended for inclusion. A lack of excessive daytime sleepiness despite a reduction in total sleep time is a supportive diagnostic criterion for RLS,[6] but with low diagnostic value and it is therefore not included in the recommendations.

In both humans and rodents, the preferred method for assessing sleep is polysomnography (PSG), as accelerometry-based assessments provide less precise estimates of sleep continuity and duration. In humans, this may be complemented by the multiple sleep latency test (MSLT) and the maintenance of wakefulness test (MWT) during the daytime. In rodents, PSG is often complemented by video recordings. The requisite recording duration must include the main sleep period (ie, lights-on period in rodents), and ideally exceed 24 hours. In rodents, the standard minimal PSG montage to assess sleep macroarchitecture consists of at least one implanted electroencephalography (EEG) lead (eg, frontoparietal ipsilateral

differential derivation) and simultaneous measurement of EMG derived from nuchal muscles.

Although less precise, the sleep–wake state may also be inferred from rest–activity signals detected by a diverse array of non-invasive (video tracking, cage floor piezoelectric sheets, infrared or laser beam breaks) or invasive (implanted accelerometers or activity telemeters, or neck or limb EMG recordings) activity monitors (see above). Non-invasive techniques can be used for extended periods and may help reduce animal suffering while allowing for adequate sample sizes and statistical power. These techniques have been validated against PSG for the evaluation of murine sleep, but agreement is greatest for relatively long bouts of sleep and wakefulness >30–40 seconds.[23,24] Overall estimates of sleep time/continuity may be less accurate due to short sleep bouts and sleep fragmentation compared to vPSG.[25,26] Another limitation concerns the inaccuracy in discriminating between non-REM sleep and REM sleep, although this may be partially mollified with technical refinements.[27] A more recent approach to activity-based sleep assessment relies upon whole-body plethysmography,[28] which combines gross movement detection with sleep–wake state-specific temporal dynamics of breathing.

The recommended endpoints for assessing sleep with vPSG in rodent models of RLS are a delay to sleep onset, a decrease in sleep bout length, more frequent transitions between different wake–sleep states, and a decrease in total sleep time during the light period. Given the distribution of rat and mouse sleep in multiple short episodes, each lasting seconds to a few minutes without a consolidated daily sleep period, the task force recommended assessing sleep latency during the symptomatic circadian period, after a short period (10–20 minutes) of sleep deprivation by gentle handling.[29]

Clinical Features Concerning Responses to Pharmacological Interventions and Iron Deficiency

Both the signs and symptoms of RLS can be reduced or increased by a variety of pharmacological compounds with unique mechanisms of action (Table 4). These clinical features of RLS can be leveraged for translational research in that they provide relatively selective means to rescue or induce the RLS-like phenotype in animal models. The effect of the pharmacological interventions listed below may be tested by using any method and outcome measure recommended so far. However, it should be taken into account that dopaminergic modulation has its most remarkable impact on sensory symptoms and motor signs like PLM, while opioids are similarly effective on sensory symptoms, less effective on PLM, and more effective on sleep disturbances.[30]

Interventions Leading to Improvement of RLS-Like Behavior

Low-dose dopamine agonists generally improve symptoms and signs (eg, PLMS) immediately, although a small percentage of patients receive no benefit. Among dopaminergic treatment options, the clinicians suggested levodopa and the dopamine receptor agonists pramipexole, ropinirole, and rotigotine.

Opioids provide relief for RLS as well as for many other pain conditions. Despite the lack of specificity, the clinicians considered that symptomatic improvement with opioids generally

supports the diagnosis of RLS. The suggested opioids are oxycodone and methadone, which are the most studied and are relatively selective mu receptor agonists. Importantly, methadone has recently been shown to have a unique pharmacodynamic profile compared to morphine-like (including oxycodone) and fentanyl-like compounds, in that methadone does not activate the dopaminergic system.[31]

Improvement of RLS symptoms may occur with iron supplementation. Iron supplementation would be useful for evaluating models in which RLS-like phenotypes emerge in association with iron deficiency. However, there is insufficient information available to issue recommendations on the dietary iron concentration and time of onset and duration of the dietary iron supplementation for rodent models. The task force did not reach a consensus on the diagnostic value of checking for RLS symptom and PLMS improvement with $\alpha_2\delta$-ligands such as pregabalin and gabapentin enacarbil, despite their wide use in clinical practice.

Interventions Leading to Worsening of RLS-Like Behavior

Dopaminergic Treatments.—RLS symptoms typically worsen upon withdrawal of dopaminergic treatment as well in smaller subgroups of medication-naïve and medicated patients treated with dopamine antagonists. D2-D3 receptor antagonists might deepen sleep or have a neutral effect on sleep. Neuroleptics, in particular early-generation drugs such as haloperidol, are well-known inducers of RLS by way of their antihistamine or antidopaminergic properties. In one pioneering attempt, haloperidol was used to induce RLS in rats with inconsistent results.[32] Metoclopramide, which is a dopamine antagonist anti-nausea drug, has also been reported to rapidly induce a worsening or appearance of RLS symptoms.[33] To induce a worsening of symptoms, the task force recommended the use of dopamine antagonists with high selectivity for D2-like receptors, in particular, D3 subtype receptors.[34]

Other Drug Treatments.—In some patients, RLS symptoms may worsen with histamine receptor antagonists, selective serotonin reuptake inhibitors (SSRI), or serotonin-norepinephrine reuptake inhibitors (SNRI). Among the antihistaminergic drugs, diphenhydramine, which often worsens RLS, can be considered for experimental interventions to worsen RLS-like behavior. Antidepressants that modulate serotonin and norepinephrine activity, such as SSRI (eg, escitalopram), SNRI (eg, venlafaxine),[35] and noradrenergic and specific serotonergic antidepressants (NaSSA, eg, mirtazapine)[36] can also be considered, also to induce the PLMS component. Moreover, other compounds with similar binding properties may be considered, including experimental molecules not available on the market, as long as they cross the blood–brain barrier. However, the worsening of RLS with these agents is not universal and may vary from patient to patient and should therefore be interpreted with caution in animal research.

Iron Deficiency.—History of iron deficiency is a feature often encountered during the diagnostic assessment of RLS,[6] although the effect of iron deficiency on PLMS has yet to be ascertained. Accordingly, the clinicians proposed the occurrence or worsening of symptoms with a deficiency in mobilizable stores of iron as a new clinical feature of RLS. Importantly,

the mechanism leading to the occurrence or worsening of RLS is believed to be the specific deficiency of brain iron,[37] whereas there seems to be less of a contribution of peripheral iron and iron stores. The suggested clinical methods of evaluation included measurements of serum and cerebrospinal fluid ferritin levels and serum transferrin saturation, and transcranial sonography of the substantia nigra. Recent evidence indicates that post-weaning diets with iron as low as 9 parts per million (ppm) for 5 months are insufficient to induce brain stem iron deficiency in C57Bl6/J mice,[38] whereas diets with iron 3–5 ppm for 3 months suffice for some brain areas for most but not all BXD strains of mice.[39] On this basis, the task force recommended protracted (at least 90 days) dietary treatment with a severely iron-deficient diet (iron content 3–5 ppm) to induce or worsen RLS-like behavior. Due to the resistance of the adult rodent brain to nutritionally induced iron deficiency, it is essential to start the iron-deficient diet at weaning. Other methods such as repeated blood withdrawal might also be considered if first validated for induction of brain iron deficiency in rodents. Ideally, at the end of the iron-deficient diet period, brain iron deficiency should be confirmed in anatomical areas implicated in RLS pathophysiology, including the spinal cord (preferably the thoracic and lumbosacral components), ventral striatum/nucleus accumbens, putamen, and ventral midbrain.

Supportive Clinical Features Concerning RLS History, Sex, and Age

RLS History—The presence of RLS among first-degree family members is considered valuable in confirming a clinical diagnosis. Genetic screenings indicate that genetic factors are important contributors to RLS,[40] also demonstrated by a very high RLS prevalence in first-degree relatives of RLS patients.[41] Direct translation of this clinical feature to inbred strains of rats and mice would be meaningless. However, knockout murine models for genes associated with human RLS have been generated and are informative.[42, 43] Knockout rat technology is also being increasingly developed, and differences in RLS-like behavior among rodent strains with different genetic backgrounds will be interesting to explore in greater detail.

Sex—The prevalence of RLS is twofold greater in women than in men.[44] The origin of this difference remains enigmatic. The clinicians suggested that sex be included as a new clinical feature but acknowledged that it is of little diagnostic value. On this basis, the task force recommended that experiments on RLS models be performed on rodents of both sexes. Both the parity of the dams and the estrous cycle phase,[45] with associated hormonal fluctuations, should be taken into account as potential confounders.

Age—The prevalence of RLS increases until the age of 60–70 years. However, age alone is of little diagnostic utility. It should also be highlighted that RLS might occur in children, that its relationship with age is still unclear in the Asian population, and that it is very frequent among pregnant women. The task force suggested that basic scientists favor the use of mature or old adult rodents for their experiments and, where practical, evaluate age-dependent increases in RLS-like behaviors. One study[32] has reported leg movement activity in healthy old rats. Frailty, comorbidity, and age-related sleep changes should be taken into account when studying old rodents. Recognizing that many of these factors may strongly depend on genetic background, the task force refrained from recommending fixed age

ranges for the study of mouse and rat RLS models. Researchers should consult the available evidence on approximate human age equivalences in their model of choice when designing their experiments.

While human RLS is often chronic, intermittent or transitory RLS is also common, and periods of spontaneous remission or improvement may occur—even subclinically—especially early in the condition. The RLS natural history is of low diagnostic utility and difficult to translate directly to animal model research.

Discussion

The IRLSSG task force generated consensus guidelines for assessing RLS-like behavior in rodent models. To assess the core subjective symptoms of RLS, surrogate behavioral measures were recommended. Activity-based techniques were recommended in addition to gold standard vPSG approaches to assess sleep disturbances and PLMS, which are objective features that are highly comorbid with RLS. Additionally, the task force recommended specific pharmacological interventions or induction of iron deficiency to rescue or worsen the RLS-like behavior in rodents.

These guidelines are meant to expand scientific inquiry intent on advancing the mechanistic understanding of RLS and developing new therapies. Research groups already active in the field may use these guidelines to standardize their animal experiments. This will make behavioral data between different groups and different proposed RLS models easier to compare, ultimately helping refine experimental procedures and avoid unnecessary duplication of experiments. These guidelines will also offer guidance to new research groups when planning their experiments to enter the field of RLS animal research.

The task force focused these guidelines on laboratory rats and mice. Rodent models are valuable due to cost-effectiveness and well-understood genetics and neuroanatomy. They remain essential for the testing of pathophysiological hypotheses and preclinical testing of novel treatments, also owing to the application of neural modulation techniques such as opto- and chemogenetics and to the availability of genetically modified strains. The task force endorses the principles of the 3Rs (Replacement, Reduction, and Refinement, cf. https://www.nc3rs.org.uk) for animal experimentation, and considers it mandatory that all animals used in research are treated humanely and compassionately.

These guidelines focus solely on the face validity of RLS animal models. In addition, it is vital to consider construct validity in assessing a potential RLS animal model.[46] Attempts to induce RLS-like behavior in animals have included lesioning as well as pharmacological, genetic, and nutritional modifications. The construct validity of these different approaches depends on our current understanding of RLS pathophysiology. The ability to prove construct validity is limited by the current status of our understanding and thus is continually evolving.

The main strength of these guidelines lies in the fact that the task force was composed of leading RLS clinical experts and basic scientists currently involved in RLS animal science.

In particular, the methods were designed to achieve a directional information flow from the clinicians to the basic scientists.

The recommendations made by the task force are limited by the lack of an abundance of rodent data available relating to most phenotypes discussed. Due to this limitation, the task force refrained from recommending cutoff values for any outcome measures and instead decided to only indicate the expected direction of change (endpoint) in an RLS animal model compared to unaffected controls. The task force also chose to recommend an array of methods that it considered as suitable for animal model translation of human RLS features, but refrained from proposing fixed hierarchies of methods. The choice of method should be dictated by the individual experimental design. As for any set of guidelines, updates should occur when sufficient additional clinical and experimental data become available. To facilitate this aim, the task force encourages researchers to include video clips of recorded RLS-like phenotypes in their research reports, following the example of reference.[19] A collective video repository of RLS-like behaviors recorded in different laboratories and rodent models is presently lacking and would be key for the future refinement of these guidelines.

In conclusion, we present the first methodological guidelines for modeling RLS-like behavior in rodents. We hope these guidelines will foster the development of novel and more refined and comparable animal models of RLS, with the intent of advancing our understanding of RLS pathophysiology and, ultimately, of improving the range, efficacy, and side effect profiles of therapeutic options.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1.

Terminology and definitions

Term	Definition
Clinical features	Clinical aspects relevant for the diagnosis of RLS in humans
Face validity	How closely the animal model reproduces the RLS clinical features
Construct validity	How well the mechanisms used to induce the RLS clinical features in the animal model reflect the currently understood RLS pathophysiology in humans
Predictive validity	How well the animal model allows extrapolation of results (including drug screening for potential therapeutics) to other species, such as humans
Method	The clinical/experimental procedure used by investigators to best assess a given aspect of the human/animal phenotype
Outcome measures	The variables to be measured by the methods
Endpoint	The direction of change (improvement or worsening) of a given outcome measure

RLS, restless legs syndrome.

TABLE 2.

Recommended animal model translations of restless legs syndrome (RLS) essential criteria and related clinical features

	Clinical features	Recommended animal model methods	Recommended animal model outcome measures (expected endpoints)
1	An urge to move the legs usually but not always accompanied by, or felt to be caused by, uncomfortable and unpleasant sensations in the legs	Activity monitoring for assessing urge to move[a]	• Increase in total activity (movements/distance traveled) in the home cage • Increase in EMG activation events/h in a restrainer • Increase in number of tibialis anterior EMG bursts/h during wakefulness in home cage • Decrease in total duration of periods of very low or no activity in the home cage
		Video recordings	• Increase in number of events of limb activity resembling leg rubbing, kicking, flexing, stretching, or fidgeting
2	The urge to move the legs and any accompanying unpleasant sensations begin or worsen during periods of rest or inactivity such as lying down or sitting	Video recordings	• Increase in general activity in the home cage (including locomotion, rearing, grooming, limb stretching)
		Activity monitoring[3]	• Decrease in time spent at rest in the home cage • Increase in EMG activation events/h in a restrainer
2a	The urge to move the legs and any accompanying unpleasant sensations during rest occur or are worse in the transition from wake to the main sleep period, and potentially extend to wakefulness bouts throughout the main sleep period	Activity monitoring[8]	• Increase in total activity (movements/distance traveled) in the home cage occurs or is maximal in last part of the active period and during activity bouts in the rest period
		Video-PSG	• Increase in EMG activation events in the home cage occurs or is maximal during wakefulness in the last part of the active period and in the rest period
3	The urge to move the legs and any accompanying unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, at least as long as the activity continues	Activity monitoring[a]	• Decrease in the median duration of rest episodes when in home cage
3a	Behavior manifestations of lower extremity discomfort	Video recordings	• Increase in the ratio of time with hindlimbs vs. forelimbs at rest
		Activity monitoring[a]	• Increase in hindlimb vs. forelimb EMG activity
3b	The distal predominance of sensory symptoms: symptoms are worse in the legs (ie, from knee to ankle) compared to the rest of the body	Video recordings	• Increase in frequency of episodes of touching/rubbing/licking hindlimbs vs. forelimbs • Increase in frequency of episodes of hindlimb licking at the level of femur vs. at the level of tibia and foot
4	The urge to move the legs and any accompanying unpleasant sensations during rest or inactivity only occur or are worse in the evening or night than during the day	Activity monitoring[a]	• General activity in the home cage (increase vs. controls greater at the end of the dark period and during the light period than at the beginning of the active period)
		Video-PSG	• Number of tibialis anterior EMG bursts/h during wakefulness in the home cage (increase vs. controls greater at the end of the dark period and during the light period than at the beginning of active period)
4a	Circadian pattern	Activity monitoring[a] with measurement of a circadian marker (eg, core body temperature) in LD conditions, then switch to DD	• Phase-shift between the rhythm of incidence of tibialis anterior EMG bursts (classified as a function of their intermovement interval) and rhythms of the circadian marker during LD and DD (RLS-like phase shift in LD, preserved in DD)
		Video-PSG with measurement of a circadian marker (eg, core	• Phase-shift between rhythm of incidence of TA EMG bursts (classified as a function of their intermovement interval) and rhythms of the circadian marker during LD and DD (RLS-like phase shift in LD, preserved in DD)

	Clinical features	Recommended animal model methods	Recommended animal model outcome measures (expected endpoints)
		body temperature) in LD conditions, then switch to DD	
5	The occurrence of the above features is not solely accounted for as symptoms primary to another medical or a behavioral condition (eg, myalgia, venous stasis, leg edema, arthritis, leg cramps, positional discomfort, habitual foot tapping)	Exclude myopathy, neuropathy, severe arthritis with pathological assessment. Demonstrate at least partial phenotype rescue with pharmacological treatment (cf. Table 4)	

EMG, electromyography; LD, light-dark cycle; DD, constant darkness conditions; PSG, polysomnography; RLS, restless legs syndrome; TA, tibialis anterior.

[a]Activity monitoring methods include, but are not limited to, video tracking, piezoelectric floor sensor, infrared or laser beam grids, implanted accelerometers or activity telemeters or voluntary running wheel, and neck or limb EMG recordings (not suited for the evaluation of distance traveled, but the only method suited for assessment of periodic leg movements, for application in a restrainer, and for hindlimbs vs. forelimb activity).

TABLE 3.

Recommended animal model translations of restless legs syndrome (RLS) clinical features related to periodic limb movements and sleep

	Clinical features	Recommended animal model methods	Recommended animal model outcome measures (expected endpoints)
6	Periodic limb movements: the presence of PLMS or PLMW at rates or intensity greater than expected for age or medical/medication status	Video-PSG	• Increase in number of TA EMG bursts/h during wakefulness or sleep as a function of their decreased inter-movement interval
7	Sleep disturbance (reduction in total sleep time, long sleep latency, more frequent sleep state transitions/fragmented sleep)	Activity monitoring	• Decrease in time spent at rest during the light period (decrease) • Increased transitions/h from activity to sustained rest and vice versa
		Activity monitoring after sleep deprivation	• Increased latency to sustained rest
		Video-PSG	• Decreased total sleep time during the light period • Decreased sleep bout length • Increase in transitions/h between different wake-sleep states
		Video-PSG after sleep deprivation	• Increased sleep latency

PLMS, presence of periodic leg movements in sleep; PLMW, presence of periodic leg movements in resting wake; PSG, polysomnography; TA, tibialis anterior; EMG, electromyography.

TABLE 4.

Recommended animal model translation of restless legs syndrome (RLS) clinical features concerning responses to pharmacological interventions and iron deficiency

	Clinical features	Recommended animal model methods	Recommended animal model outcome measures (endpoints)
8	Improvement in symptoms and PLMS at least initially with dopaminergic treatment	Administration of dopamine receptor agonists or levodopa	• Rescue of CFs (CF7 possibly excepted)
9	Improvement of symptoms with opioids	Treatment with opioid mu receptor agonists (eg, oxycodone, methadone)	• Rescue of CFs (CF6 possibly excepted)
10	Rapid worsening or reappearance of symptoms after dopaminergic treatment withdrawal (except during a clear augmentation phenomenon)	Short term (eg, 4 drug half-lives) withdrawal of dopamine receptor agonists or levodopa	• Aggravation of CFs (CF7 possibly excepted)
11	Worsening of symptoms with dopamine receptor antagonists	Treatment with D2 or D3 dopamine receptor antagonists	• Aggravation of CFs (CF7 possibly excepted)
12	Worsening of symptoms with histamine receptor antagonists	Treatment with H1 histamine receptor antagonists that cross the blood-brain	• Aggravation of CFs (CF6 possibly excepted)
13	Worsening or appearance of symptoms/PLMS with SSRIs/SNRIs	Treatment with SSRI (eg, escitalopram) or SNRI (eg, venlafaxine)	• Aggravation of CFs
14	Symptoms get worse or may only occur with iron deficiency	Iron-deficient diet (<5 ppm iron starting from weaning, check for effectiveness on brain structures of interest)	• Aggravation of CFs, particularly increased activity, decreased resting in the last part of the active period in the home cage (CF6 possibly excepted)
15	Symptoms improve with iron treatment for conditions with iron deficiency	Iron-sufficient diet. Check for effectiveness on iron content of neural structures of interest	• Rescue of CFs, particularly increased activity and decreased resting in the last part of the active period in the home cage (CF6 possibly excepted).

CF, clinical feature; PLMS, periodic limb movements of sleep; SSRI, selective serotonin reuptake inhibitor; SNRI, selective serotonin-norepinephrine reuptake inhibitor; ppm, parts per million.