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CLINICAL REVIEW

Restless legs syndrome: Clinical changes in nervous system excitability at the spinal cord level

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SUMMARY

Restless legs syndrome (RLS) is a complex multifactorial disorder whose aetiology has yet to be fully elucidated. Some of the features of RLS, such as processing of sensations and activation of movement, may result from a dysfunction in spinal processing giving rise to a state of spinal hyperexcitability. In the current article we review studies investigating spinal excitability in RLS patients looking specifically at electrophysiological studies of spinal activity, sensory evaluations, and spinal reflex studies. Increased spinal excitability has been shown in RLS patients based on the combined data from electrophysiological studies. Results from studies assessing sensory evaluations in RLS patients show enhanced spinal processing of nociceptive inputs possibly due to central sensitisation. However, not all sensory modalities demonstrate an increase in sensitivity. An increase in nervous system excitability would result in an increase in reflex responses in RLS patients however the data from reflex analyses in RLS patients has failed to consistently show this expected result. Overall changes to RLS spinal excitability have been demonstrated though these changes might be heterogeneous as not all afferent input appears to be affected in the same manner. There may be phase-dependent and modality-dependent alterations in spinal excitability suggesting that the theory of absolute spinal hyperexcitability in RLS patients' needs to be reconsidered.

Introduction

Restless legs syndrome (RLS) is a disorder presenting with both sensory and motor symptoms, which are often manifest as an urge to move and periodic limb movements (PLM) respectively [1]. As the spinal cord is the site of sensory input and motor output it has been considered that the aetiology of RLS may be due to dysfunctions in the spinal cord leading to hyperexcitability [2]. It is possible that RLS patients have reductions in descending spinal inhibition due to subcortical and/or intra-cortical dysfunction [3]. The spinal cord could also be the primary site of dysfunction in RLS. Loss of supra-spinal inhibitory influence on the spinal cord or an increase in spinal excitability are both plausible candidates for RLS symptoms and PLM.

Considerable research has been conducted on spinal excitability in RLS patients. This review will discuss the salient points emanating from these investigations looking specifically at electrophysiological studies of spinal activity, sensory evaluations, spinal reflex studies, and possible neurochemical mechanisms for changes in spinal excitability in RLS patients. It should be noted that this review is focused purely on possible neural circuit abnormalities that result in RLS symptoms and does not discuss the modulating effect of pharmacological treatments on these symptoms.

Electrophysiological studies of spinal cord and peripheral nerve activity in RLS patients

An electrophysiological method of evaluating motor and cutaneous nerve function is to assess the F-wave and the cutaneous silent period (CuSP) respectively. Elicitation of the F-wave is by electrical stimulation of the distal portion of a motor neuron. Measurements from the F-wave assessment are; F-wave duration and the ratio of F-wave to compound muscle action potential (CMAP) duration, which are indices of motor neuron function [4].

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Abbreviations

CPG	Central Pattern Generator
CMAP	Compound Muscle Action Potential
CPT	Current Perception Threshold
CuSP	Cutaneous Silent Period
EMG	Electromyography
FWR	Flexor Withdrawal Reflex
Hmax	H-reflex maximum amplitude
H-reflex	Hoffman reflex
Ih	Hyperpolarization-activated currents
Mmax	M-wave maximum amplitude
NREM	Non-rapid eye movement
PLM	Periodic Limb Movements
PLMS	Periodic Limb Movements during sleep
QST	Quantitative Sensory Test
RLS	Restless Legs Syndrome
rTMS	Repetitive Transcranial Magnetic Stimulation
tsDCS	Transcutaneous Spinal Direct Current Stimulation
VAS	Visual Analogue Scale

The CuSP is a brief interruption of voluntary muscle contraction due to electrically activating a cutaneous nerve allowing for the evaluation of cutaneous nerve function and overall sensorimotor integration [5] which may be dysfunctional in RLS patients.

To date there have been three studies that have investigated the F-wave [4,6,7] and five studies that have assessed CuSP in RLS patients [4,6–9]. Two of the studies have demonstrated an increased F-wave duration and F-wave/CMAP duration ratio in RLS patients when compared to control participants [4,6] while the third study only reported on F-wave latency [7]. All three studies did not observe any differences in F-wave latencies between RLS patients and control participants. The increased F-wave durations suggest that there is possibly increased excitability of motor neurons in RLS patients. Furthermore, studies have noted decreased *tibialis anterior* CuSP duration in RLS patients [4,7,8]. It has been postulated that alterations in spinal interneuron function may be the mechanism for the decreased CuSP duration in RLS patients [4]. However, one study did not show changes in *tibialis anterior* CuSP duration in RLS patients [6] while another study demonstarted increased *extensor halluces brevis* CuSP duration in RLS patients [9]. The latter study was the only one to assess CuSP in the *extensor halluces brevis* muscle [9]. The difference in results regarding the CuSP duration could therefore be as a result of differences in muscle responses or methods of stimulation (sural nerve stimulation versus hallux ring electrodes). The opposing results may also be due to differences in the early compared to late stages of RLS [4] as all of the studies that observed a decrease in CuSP duration consisted of recently diagnosed RLS participants [4,7,8]. In comparison, RLS patients in the studies that did not report a decrease in CuSP duration study had a mean RLS duration of 9.1 y [6] or did not report on the RLS duration [9] and may have had patients with more advanced RLS. Further investigations are therefore required to confirm if changes in CuSP duration is a consistent observation in RLS patients and if so if this is due to changes in spinal interneuron function. The decreased CuSP duration [4,7,8] could be a result of the increased excitability of motor neurons, as shown by the increased F-wave duration and F-wave/CMAP duration ratio in RLS patients, or as a result of a decrease in afferent neuron sensitivity. Future studies should furthermore assess cortical excitability in conjunction with CuSP to determine if the decreased CuSP duration is due to changes in cortical excitability, spinal excitability or both. CuSP may also be influenced by supraspinal effects [5] and thus cortical contributions to changes in CuSP cannot be ruled out. Overall data from transcranial magnetic stimulation studies in RLS patients support the theory of increased cortical excitability in RLS which is possibly due to alterations of cortical inhibitory circuits [3]. Alternatively, increased cortical excitability may be due to prolonged afferent activity caused by the constant unpleasant sensations that are described by RLS sufferers. It has been demonstrated that prolonged afferent activity causes increased excitability of the motor cortex [10]. Therefore alterations in CuSP variables may be due to changes in cortical contributions however, increased cortical excitability might be merely an epiphenomenon to an increase in afferent processing due to hyperexcitability of the spinal cord.

In support of a possible increase in motor neuron excitability leading to a decreased CuSP duration in RLS patients, a recent study reported hyperexcitability in motor axons of RLS patients compared to healthy control participants [11]. A greater accommodation to hyperpolarisation in motor axons of RLS patients, compared to those of control participants, was observed from a threshold tracking technique which involved threshold stimulus changes, in axons of the median nerve, following depolarising and hyperpolarising conditioning currents [11]. The authors theorise that this motor axon hyperexcitability in RLS patients is due to increased hyperpolarization-activated currents (Ih) [11]. In contrast, no differences were reported in sensory axon excitability between RLS patients and control participants [11].

Due to the theory of RLS symptoms having a spinal origin transcutaneous spinal direct current stimulation (tsDCS) has been proposed as a treatment for RLS patients [12]. tsDCS is a non-invasive technique to alter spinal excitability by applying a weak current over the thoracic spinal cord via surface electrodes [12]. The effects of tsDCS on RLS symptoms have been assessed using a visual analogue scale (VAS), as a subjective measure of symptom severity [12]. In the same study H2/H1 ratio of the soleus Hoffman reflex (H-reflex) provided an objective assessment of spinal excitability to determine if the effects of tsDCS were mediated through changes in spinal excitability. RLS patients had increased H2/H1 ratios compared to control participants at baseline [12] and following tsDCS treatment, a decrease in H2/H1 ratios was noted in RLS patients for at least 15 min after stimulation. Furthermore, there was a significant decrease in subjective symptom severity following tsDCS treatment in RLS patients as measured using the VAS [12]. The effect of tsDCS on the severity of symptoms in RLS implicates spinal cord hyperexcitability in generating the urge to move in RLS patients. The important role of spinal cord hyperexcitability is strengthened by the fact that transcranial direct current stimulation of the somatosensory cortex, as opposed to stimulation of the thoracic spinal cord, had no effect on RLS symptom severity [13]. However, low-frequency repetitive transcranial magnetic stimulation (rTMS), another non-invasive stimulation technique, has been reported to improve subjective reporting of sleep initiation and maintenance in RLS patients [14]. The authors theorised that the benefits of rTMS on RLS may be due to rTMS activating descending dopaminergic impulses [14] which could be preventing abnormal somatosensory processing at the spinal level. Other studies assessing the effects of low-frequency rTMS on chronic insomnia [15] and epilepsy [16] have also reported improvements in sleep parameters. Thus the improvement in sleep initiation and maintenance in RLS patients may be due to independent effect of rTMS on sleep and unrelated to the pathophysiology of RLS. Thus, future investigations into rTMS as a treatment for RLS should evaluate improvements in other RLS symptoms, such as IRLS score and PLM during sleep (PLMS).

tsDCS therapy affects spinal sensory and motor processing. The effect of tsDCS on nociception has been demonstrated by increases in pain tolerance following tsDCS treatment [17]. In addition to effects on afferent inputs, tsDCS also reduces post activation depression of the H-reflex [18] and depresses lower limb flexion reflexes [19]. Thus, relief from sensory and motor RLS symptoms following tsDCS therapy may be due to tsDCS modulating spinal excitability however further investigation in RLS patients is required.

The presence of PLM is not a definitive criterion of RLS however approximately 80% of RLS patients experience PLM [20]. Thus exploring PLM from a spinal cord perspective is warranted. The presence of PLMS has been speculated to be a consequence of the interruption of descending supraspinal inhibition during sleep [21]. Studies on patients in states of severe disturbance to supraspinal input (spinal cord injury patients and patients under spinal anaesthesia) have found the presence of PLM type movements, similar to those seen in RLS patients, which suggest a spinal cord origin of these involuntary movements [22–25]. In addition supporting a spinal origin of PLM is the concept that PLM are possibly generated by the spinal central pattern generators (CPG) for gait. CPGs generate rhythmic, repetitive and stereotyped movements [26] which are characteristic traits of PLM. Increased excitability of the spinal cord could possibly trigger spontaneous movements such as those seen in PLM. CPGs can also act independently of supraspinal control [27]. Furthermore sensory feedback plays a major role in modulating CPGs, thus central sensitisation of the spinal cord may lead to abnormalities in the processing of sensations causing the release of CPG motor programs resulting in the urge to move and PLM. Additionally the cortical involvement in PLMS, PLM while awake, and voluntary dorsiflexion in RLS patients has also been assessed looking at spontaneous electroencephalographic oscillations [28]. Event-related desynchronisation occurred before PLM while awake and voluntary dorsiflexion; however no event-related desynchronisation was apparent before PLMS [28].

Further supporting the theory of a spinal origin of PLM is the similarity in movement and muscle recruitment patterns between PLM, the spinal flexor withdrawal reflex (FWR) and the Babinski reflex. Therefore PLM may have spinal circuitry in common with the Babinski reflex and the FWR. Video image analysis in the 1980s revealed that PLMS resembled the Babinski sign [29]. The importance of the similarity between PLM and the Babinski sign is that clinically, the presence of the Babinski sign may be indicative of upper motor neuron lesions. Disorders involving damage to upper motor neurons, that isolate the spinal cord from supraspinal inhibitory control, are classical examples of spinal hyperexcitability [30]. More recently, similarities between the FWR and PLM have been demonstrated suggesting that the two may share a common, spinal generator [31]. The anterior tibialis muscle is recommended as the muscle of choice for EMG recordings to detect and quantify PLM, as dorsiflexion of the ankle (a result of anterior tibialis muscle contraction) is one of the defining features of PLM [32]. In addition to the anterior tibialis muscle initiating the PLM motor sequence, other muscles involved in the FWR motor pattern (gastrocnemius, biceps femoris, rectus femoris and extensor digitorum brevis) have also been shown to begin the PLM muscle contraction sequence [33,34]. These results suggest that there is no cortical involvement in PLM supporting the hypothesis of a spinal generator of PLM.

The combined data from the electrophysiological evaluations of spinal cord activity in RLS patients (summarised in Table S1) indicate that the pathogenesis of RLS may involve spinal cord hyperexcitability. Furthermore the spinal hyperexcitability may be a result of alterations in afferent neuron, motor neuron, or interneuron function. However there are methodological concerns regarding the studies assessing spinal excitability of RLS patients. None of the studies corrected for possible changes in spinal excitability due to the effects of sleep loss or the constant spinal activation during sleep due to PLMS. The increased spinal activation during sleep in RLS patients may have an effect on the spinal circuitry leading to the increased spinal excitability seen in RLS patients. In addition, some of the patients in the study exploring the effects of tsDCS on RLS symptoms [12] were taken off dopaminergic agonists the day before tsDCS testing. Dopaminergic agonists are known to have an effect on both cortical and spinal excitability which could be responsible for the differing responses seen in RLS patients compared to control participants. Furthermore the studies were performed at different times of day with a large number of studies not even mentioning the time data was collected or if the time remained constant for all patients. For comparison of results between studies it is essential that time of day is taken into account due to the circadian nature of RLS symptoms. Nevertheless, to determine the full pathophysiological mechanism of spinal hyperexcitability in RLS patients requires further electrophysiological investigation. As RLS is characterised by sensory symptoms a large focus of previous studies has been on the evaluation of spinal sensory pathways in RLS patients.

Sensory evaluations in RLS patients

A key diagnostic feature of RLS is the urge to move accompanied by unpleasant sensations [1]. Research has subsequently focused on spinal sensory pathways in RLS patients, in particular pain pathways, to elucidate the mechanism responsible for these unpleasant sensations. As stated previously, there is evidence to indicate that there is a state of spinal hyperexcitability in RLS patients and this could result in hyperalgesia. Hyperalgesia is defined as an increased sensitivity to noxious stimuli. Nociceptive pathways in RLS patients have been assessed, in the evening and the morning, with the use of a punctuate mechanical stimulus [35]. This type of mechanical stimulus activates nociceptive high threshold A-delta fibres and tests for the presence of hyperalgesia [35]. The authors also investigated the presence of allodynia by activating low threshold mechanoreceptive A-beta fibres using a moving gentle stimulus [35]. Allodynia is a phenomenon where normally non-noxious stimuli are perceived as painful. RLS patients had increased pain ratings in response to punctuate mechanical stimuli when compared to healthy control participants [35]. Moreover, the increased pain ratings in RLS patients in comparison to control participants were noted in both the morning and the evening, thus excluding the possibility of a circadian influence. These findings show that there is a general state of a degree of hyperalgesia in RLS patients. As no allodynia was reported in the RLS patients, the authors concluded that the hyperalgesia was likely mediated by central sensitisation of high threshold A-delta fibre inputs [35].

The presence of hyperalgesia in RLS patients has been further established using the quantitative sensory testing (QST) protocol [36,37]. The QST protocol incorporates thermal and mechanical stimuli to assess thermal, mechanical and noxious sensitivity and thresholds [38,39]. The QST also evaluates the presence of allodynia. The QST protocol has been performed, during the symptomatic period, on the dorsum of the feet of RLS patients and healthy control participants [36]. RLS patients had decreased pain thresholds to both pressure and mechanical stimuli when compared to the healthy control participants. These data indicate that there was hyperalgesia to blunt pressure and pinprick pain in

RLS patients. In addition, no allodynia was reported in the primary RLS patients [36].

The other study using the QST protocol on RLS patients assessed in the morning, during the asymptomatic period, exhibited decreased mechanical pain thresholds in RLS patients however significantly increased mechanical detection thresholds compared to control participants [37]. Furthermore, allodynia was not seen in the RLS patients or healthy control participants [37]. The results from the studies using the QST protocol are in agreement in that both studies indicate the presence of hyperalgesia in RLS patients and the absence of allodynia [36,37]. In addition, RLS patients displayed an increase in mechanical detection threshold, which is an indicator of a reduced perception of mechanical stimuli (mechanical hypoesthesia) [37]. The difference in results regarding pain thresholds and mechanical detection thresholds may indicate a more complex spinal cord dysfunction in RLS patients beyond only spinal hyperexcitability. With a global state of spinal hyperexcitability a decrease in mechanical detection thresholds, in addition to the decreased pain thresholds, would present in RLS patients. However based on the results of the QST protocol in RLS patients this may not be the case.

A study in 2011 looked at differences in pain thresholds between RLS patients and control participants in the early afternoon by means of pressure and heat stimuli [40]. Consistent with the results of previous studies, RLS patients had lower pressure pain thresholds when compared to control participants. RLS patients also displayed greater temporal summation of heat pain [40]. Temporal summation to heat pain is a measure of central pain facilitation and evaluates the subjective pain reported following a rapid series of noxious heat stimuli of equal intensity. RLS patients reported each heat stimulus as more painful than the last, despite no change in the temperature of the stimulus [40]. Temporal summation corresponds to central sensitisation, hence these results support the hypothesis of a state of central sensitisation in RLS patients.

To evaluate potential mechanisms behind sensory threshold changes in RLS patients, small fibre neuropathy has been investigated by assessing warm and cold detection thresholds, and autonomic small fibre function [41]. RLS patients had increased warm and cold detection thresholds of the feet when compared to control participants. There were no significant differences in small fibre function between RLS patients and control participants indicating that there was no presence of small fibre neuropathy in the RLS patients. These findings indicate a central, not peripheral, cause of the increased thermal detection thresholds in RLS patients [41]. This suggests that there is no peripheral nerve damage in RLS patients and the pathophysiology of RLS likely involves alterations in central processing in the spinal cord or the brain.

The circadian variation in sensory thresholds of A-beta, A-delta and C fibres in RLS patients has recently been evaluated with the current perception threshold (CPT) test [42]. The CPT test was performed by applying currents of different frequencies to the big toe and recording when a sensation is first reported [42]. Using this method the study demonstrated a circadian variation in perception thresholds of all three fibre types in RLS patients, with decreased perception thresholds in the evening compared to the morning [42]. The only significant difference in perception thresholds between RLS patients and control participants was in evening C-fibre perception thresholds, which were lower in RLS patients [42]. Therefore RLS patients showed hyperalgesia only in small, unmyelinated nociceptive fibres. The results from this study indicate that RLS patients have increased sensitivity in afferent nerve fibres in the evening compared to the morning.

Taken together the data from studies assessing sensory evaluations in RLS patients (summarised in Table S2) indicate the presence of hyperalgesia. Several studies have noted that RLS patients have decreased pain thresholds compared to control participants. The presence of hyperalgesia in RLS patients suggests central sensitisation is present in the spinal cord and may be a potential mechanism that mediates the urge to move and the uncomfortable sensations. Importantly, RLS patients have hyperalgesia with an absence of allodynia indicating that the central sensitisation in the spinal cord is likely due to a different mechanism of other forms of central sensitisation seen in chronic pain conditions. In this regard, neuropathic pain has symptoms similar to RLS but is associated with hyperalgesia and allodynia. In terms of the circadian variation noted in the sensory pathways, RLS patients have been noted to have increased sensitivity in A-beta, A-delta and C fibres in the evening. Interestingly, hyperalgesia has been shown in RLS patients during both the symptomatic and asymptomatic periods of the disorder. These data indicate that hyerpalgesia is always present in RLS patients but the manifestation of symptoms may be subsequent to increased sensitivity in afferent fibres in the evening.

As RLS probably involves alterations in nervous system excitability the evaluation of spinal excitability and sensorimotor integration in the spinal cord of RLS patients could provide important information regarding the aetiology of RLS. A potential method for the evaluation of excitability and sensorimotor integration is through spinal reflexes.

Spinal reflexes in RLS patients

As previously discussed, the spinal cord is a probable site of the aetiology of RLS with a possible state of spinal hyperexcitability noted in RLS patients. One possible mechanism to assess changes in spinal excitability is to evaluate reflex responses. Motor responses to sensory stimuli, such as those used to elicit a reflex response, are influenced by changes in the state of spinal excitability. A reflex response involves an afferent input, spinal integration and an efferent output. The resultant reflex response therefore is dependent on the activation threshold of the afferent neurons as well as the sensitivity of the efferent neurons responsible for the motor response.

The majority of spinal reflex studies in RLS patients have assessed the FWR and the H-reflex, with one study assessing the patellar reflex in addition to the H-reflex [43] and one study assessing the crossed extensor reflex in addition to the FWR [44]. Only one study, to date, has assessed the plantar reflex in RLS patients [45]. A summary of the results from studies assessing spinal reflexes in RLS patients are shown in Table 1.

The FWR is a useful tool allowing for the elucidation of the pathophysiological mechanisms in RLS particularly considering that the sensations experience by RLS patients are often described as painful [46]. Four studies have assessed the FWR in RLS patients. RLS patients exhibit increased FWR excitability compared to control participants in three of the investigations conducted to date [31,47,48] with one study reporting no significant difference in FWR or crossed extensor reflex responses between RLS patients and control participants [44]. Therefore there is conflicting data with respect to the analyses of the FWR in RLS patients. Results from one study have demonstrated no significant differences between RLS and control groups in both EMG amplitude and latency during the FWR [44]. Consistent with those results, other studies analysing the FWR between RLS patients and healthy controls have not noted differences in EMG latency [31] and amplitude [48] of the FWR. However, one of those studies did demonstrate increased FWR responses with decreased stimulus thresholds and increased

Table 1
Summary of spinal reflex studies that compared restless legs syndrome (RLS) patients to healthy control participants.

Study	Patients (n)	Controls (n)	Time	Reflex assessed	Response in RLS patients
Bucher et al., 1996 [72]	25	15	Asymptomatic period	Soleus H-reflex	Normal
Bara-Jimenez et al., 2000 [31]	10	10	Awake: 21:30–22:30 Asleep: NREM	Flexor reflex	Increased excitability
Aksu and Bara-Jimenez, 2002 [47]	20-sRLS	20	Awake: 21:30–22:30 Asleep: NREM	Flexor reflex	Increased excitability
Rijsman et al., 2005 [50]	9-PLMD (8 RLS)	16	Late afternoon	Soleus H-reflex Vibratory inhibition of the H-reflex	Normal Decreased vibratory inhibition
Scaglione et al., 2008 [51]	7	10	AM	Soleus H-reflex Group Ib nonreciprocal inhibition	Normal Decreased Ib inhibition
Kerr et al., 2011 [43]	10 9	7 9	PM: 17:30–18:30 AM: 08:00–09:30 PM: 18:30–19:30 AM: 07:00–08:00	Peroneal H-reflex Patellar reflex	Normal No circadian variation PM decreased excitability (compared to controls and to RLS AM)
Marconi et al., 2012 [52]	9-RLS 11-sRLS	10	Afternoon	Soleus H-reflex Group Ib nonreciprocal inhibition	Normal Decreased Ib inhibition (in primary RLS only)
Heide et al., 2014 [12]	14	14	Symptomatic period (16:00–02:00)	Soleus H-reflex H2/H1 ratio	Normal Decreased inhibition
Dafkin et al., 2017 [45]	13	13	PM: 20:00–22:00 AM: 06:30–08:00	Plantar reflex	Decreased excitability (compared to controls PM and AM) PM increased excitability (compared to RLS AM)
Gunduz et al., 2017 [48]	12	17	Afternoon: 13:30–15:30	Flexor reflex	Increased excitability
Özsimsek & Koyuncuoglu, 2017 [7]	30	30	No time mentioned	Gastrocnemius H-reflex	Increased H_{max}/M_{max} ratio
Dafkin et al., 2018 [44]	12	12	PM: 20:00–22:00 AM: 06:30–08:00 PM: 20:00–22:00 AM: 06:30–08:00	Flexor reflex Crossed extensor reflex	Normal PM increased excitability (compared to RLS AM) Normal PM increased excitability (compared to RLS AM)

AM, morning; H_{max}, maximum H-reflex amplitude; M_{max}, maximum M-wave amplitude; NREM, non-rapid eye movement; PLMD, periodic limb movement disorder; PM, evening; sRLS, secondary RLS.

spatial spread (number of muscles involved in the reflex response) in RLS patients when compared to control participants [31]. Similarly, the other study showed increased durations of *tibialis anterior* muscle contraction and decreased *tibialis anterior* latency in RLS patients compared to controls, thus showing quicker and longer reflex responses [48]. It should be noted that EMG latency is dependent on stimulus intensity [31] hence making inter-group and -study comparisons difficult. A possible factor that could mask detection of a potential increase in FWR and crossed extensor reflex responses in the study that reported no differences in EMG recordings of the FWR in RLS patients compared to control participants [44] may be the large inter-individual variation observed in reflex responses. To confirm whether the FWR and crossed extensor responses are different in RLS patients when compared to healthy controls requires large scale studies that mitigate the considerable inter-individual variations in reflex responses. Collectively, from the results available to date, it is difficult to conclude if there are alterations in the FWR in RLS patients due to lack of consistency in the variables used to measure the reflex response.

Two of the studies assessing the FWR in RLS patients also assessed the FWR response during non-rapid eye movement (NREM) sleep. During sleep both the RII and RIII components of the FWR had increased elicitation thresholds in healthy control participants and not in RLS patients [31,47], which indicate that RLS patients do not display the normal increased inhibition of the FWR during sleep. As the FWR resembles the movements seen in PLM, the absence of inhibition of FWR circuits during sleep in RLS patients may be the cause of PLMS. This further supports the concept of a spinal generator for PLM. The increased FWR excitability during NREM in RLS patients could be due to the nociceptive nature of the FWR. The FWR is elicited by the activation of A-delta nociceptive fibres [49]. The presence of hyperalgesia, mediated by A-delta input [35], has been established in RLS patients and may result in the increased excitation of the FWR response seen in RLS patients.

In contrast to no difference or increased FWR responses reported in RLS patients, plantar reflex responses were significantly decreased in RLS patients compared to control participants [45]. The decreased responses noted in RLS patients with respect to the plantar reflex may be due to activation of A-beta fibres, which are not involved in the FWR. The FWR and the plantar reflex are activated by nociceptive afferent fibres (A-delta and C-fibres), but the plantar reflex is also activated by low threshold mechanoreceptive A-beta afferent fibres. Hence, a possible explanation for the decreased plantar reflex responses in RLS patients compared to control participants may be due to alterations in A-beta fibre activity in RLS patients. Another possibility is that there is an excitatory ceiling effect of certain spinal circuits in RLS patients that negatively feeds back on other spinal circuits.

The H-reflex is a reflectory response to electrical stimulation of a nerve. The measurements recorded during an H-reflex are; the maximum H-reflex amplitude (H_{max}), the maximum M-wave amplitude (M_{max}), and the H_{max}/M_{max} ratio. Majority of studies noted no significant difference in the H_{max}/M_{max} ratios between RLS patients and control participants during the *soleus* H-reflex irrespective of whether recordings were done in the symptomatic or asymptomatic periods (Table 1). Only one study has demonstrated an increase in H_{max}/M_{max} ratio of the *gastrocnemius* in RLS patients due to an increased H_{max} with no difference in M_{max} [7]. Therefore the difference in *gastrocnemius* H-reflex responses in RLS patients is likely to be as a result of modifications in afferent nerve functioning or spinal circuitry and not alterations in motor

neurons. A possible reason for the difference in H-reflex results may be the choice of muscle that was assessed with the majority of studies reporting on the *soleus* H-reflex and the study reporting the increased H_{max}/M_{max} ratio being the only study to record the *gastrocnemius* H-reflex response [7]. Overall, H-reflex testing in RLS patients indicates that functioning of Ia afferents and alpha-motor neurons are likely not altered in RLS patients and thus supports the evidence showing no peripheral nerve conduction problems in RLS.

However, RLS patients may have altered functioning of Ib afferents. A vibratory stimulus activates Ib afferent nerves which cause presynaptic inhibition of agonist alpha-motorneurons in the spinal cord. The effects of vibratory inhibition on the *soleus* H-reflex have been assessed by applying a vibration stimulus to the Achilles tendon and subsequently stimulating the H-reflex [50]. RLS patients had decreased vibratory inhibition of the *soleus* H-reflex when compared to control participants [50]. These results indicate decreased functioning of Ib afferents in RLS patients. The findings from the aforementioned study were reinforced by two additional studies in RLS patients that assessed Ib nonreciprocal inhibition of the *soleus* H-reflex [51,52]. The results of both studies were consistent in demonstrating a reduction in Ib inhibition of the H-reflex in RLS patients compared to control participants [51,52].

In addition to Ib inhibition of the H-reflex, the H2/H1 ratio has been evaluated in RLS patients. The H2/H1 ratio is the relationship between two H-reflex responses following double stimuli at varying inter-stimulus intervals, which gives an indication of spinal excitability. Increased H2/H1 ratios were noted in RLS patients compared to control participants at inter-stimulus intervals of 200–400 ms and therefore an increased facilitation of the reflex response [12]. Data from H-reflex studies in RLS patients therefore indicate that there are likely no peripheral nerve conduction problems. However, in RLS patients there is a possible alteration in spinal interneuron circuits, demonstrated by the increased facilitation (increased H2/H1 ratios), and decreased Ib inhibition.

A limitation of using the H-reflex is that an electrically induced reflex is not naturally occurring and hence the conclusions that can be drawn from these studies are limited. A biological equivalent of the H-reflex that has been assessed in RLS patients is the patellar reflex [43]. The patellar reflex is a monosynaptic stretch reflex that allows for an interpretation of the effect of the muscle spindle on the reflex response, which is bypassed during the H-reflex. RLS patients have been shown to have decreased patellar reflex responses when compared to control participants in the evening [43]. Furthermore, in the same study there were no significant differences between the H_{max}/M_{max} ratio in RLS and control participants [43]. Naturally occurring muscle spindle reflexes, like the patellar reflex, regulate motorneuron output in a phase-dependant manner (movement dependant changes in responses) [53]. Therefore, as the H-reflex bypasses the muscle spindle, the decreased patellar reflex responses, with normal H-reflex responses, in RLS patients compared to control participants may represent a phase-dependant alteration in motorneuron regulation in RLS patients.

Only three previous studies assessed reflex responses during the symptomatic and asymptomatic periods [43–45]. H-reflex responses did not differ in the morning and the evening in RLS patients. Conversely, patellar reflex responses were decreased in the evening, during the symptomatic period, compared to morning reflex responses in RLS patients [43]. In contrast, RLS patients' FWR, crossed extensor reflex and plantar reflex responses were observed to be increased during the symptomatic period compared to the asymptomatic period [44,45].

The difference in circadian variation of reflex responses in RLS patients suggest that not all spinal circuitry is affected in the same manner in RLS. The H-reflex and patellar reflex, which display no circadian variation and decreased evening excitability respectively, are mediated by proprioceptive afferents while the plantar reflex which is mediated by nociceptive and mechanoreceptive afferents and the FWR which is mediated by nociceptive afferents were both shown to have increased excitability in the evening. Therefore, a theorised mechanism for the circadian variation in spinal excitability, and by inference the symptoms of RLS, may be due to time-dependent dysfunction in nociceptive afferent circuitry. Further studies assessing RLS circadian variation of reflex responses in larger samples are necessary to provide an improved understanding of circadian changes in spinal excitability in RLS patients.

Overall data from spinal reflex analyses in RLS patients has failed to consistently show the expected spinal hyperexcitability through an increase in spinal reflex responses. Only FWR responses demonstrate increased excitability in RLS patients compared to healthy controls. H-reflex and crossed extensor reflex responses do not differ between RLS patients and control participants while patellar and plantar reflex responses are decreased in RLS patients. However, further investigation into spinal circuitry of H-reflexes demonstrated decreased Ib inhibition and increased facilitation of H-reflex responses. Little research has been done assessing circadian variation in reflex responses in RLS patients with the results to date showing no circadian variation in H-reflex responses and a circadian variation in patellar, flexor withdrawal, crossed extensor and plantar reflex responses. More research is still needed to confirm these results as well to determine if other reflex responses in RLS patients display a circadian variation.

Possible neurochemical mechanisms for changes in spinal excitability in RLS

Dopamine deficiency has been a prominent area of research in terms of elucidating the underlying causes of RLS. Dopamine has a known circadian variation, which has been demonstrated in urine, cerebrospinal fluid and plasma concentrations of dopamine. The highest concentrations of dopamine occur in the day and the corresponding nadir in late evening/night [54,55] when RLS symptoms are most severe. Therefore dopamine deficiency was seen as a likely candidate for the underlying cause of RLS and warranted further investigation in RLS patients. However, despite the initial optimism at finding a dopamine linked aetiology of RLS, evidence for the expected hypo-dopaminergic state in RLS patients has proven to be elusive. Overall results from brain imaging studies have been summarised in a recent review [56] and indicate an increase in striatal dopamine concentrations; a decrease in striatal dopamine two receptors (D2R) and decreased membrane bound dopamine transporter in RLS patients. Hence, current data supports the theory of increased dopamine presynaptically, due to a decreased reuptake of dopamine, and decreased effects of dopamine at the post-synaptic membrane due to decreased D2R. Increased dopamine concentrations have been shown to have different effects on excitatory dopamine one receptor (D1R) and inhibitory D2R expression. Increased dopamine concentrations result in a degradation of D2R and recycling of D1R [57]. Hence, the overall effect of increased dopamine concentrations may be a net increase in excitatory D1R and a decrease in inhibitory D2R at the membrane surface. These changes in dopamine receptor expression, if present in the spinal cord, may lead to a state of increased excitability which could be responsible for the generation of RLS symptoms.

A possible explanation for the positive response noted in RLS patients with respect to dopamine treatment may be changes in glutamatergic responses mediated through an effect of

dopaminergic agonists on post synaptic membrane sensitivity to glutamate. Animal studies have shown that dopamine binding to D2R decreases AMPA receptor responses [58] while dopamine binding to D1R increases NMDA evoked responses [59]. Hence, the alleviation of symptoms in RLS patients, with D2-like receptor agonist treatment, could be due to changes in sensory processing in the dorsal horn as a result of decreased glutamatergic responses. In addition, a possible mechanism of action for the symptom alleviation with α2δ ligand treatment, an alternative treatment option for RLS, is by decreasing presynaptic glutamate release which decreases post synaptic excitability and thus decreases general excitability of the nervous system. These α2δ containing calcium channels are found in the spinal cord dorsal horn [59] and the action of α2δ ligands has been demonstrated in the spinal cord [60]. Thus, the beneficial effects of α2δ ligands in the treatment of RLS may be due to a decreased release of excitatory neurotransmitters at the dorsal horn of the spinal cord [61]. Therefore, the positive effects of α2δ ligand treatment in RLS patients gives credence to the theory that spinal circuitry dysfunction mediated by inappropriate neurotransmitter release could be present in patients with RLS.

Abnormal glutamate levels have been linked to RLS, with an increase in thalamic glutamate activity noted in RLS patients compared to control participants [62]. However these findings were not duplicated in another MRI study which showed no difference in thalamic glutamate levels between RLS patients and control participants [63]. Changes in spinal glutamatergic responses may be more pronounced than changes in the brain and further investigations into the expression of spinal glutamatergic receptors and spinal glutamate concentrations are required in RLS patients.

Given the known link between RLS and iron deficiency, the relationship between glutamate and iron has also been investigated. It has been theorised that iron deficiency may produce increases in glutamate concentrations with these effects mediated through adenosine [64]. Iron deficiency has been shown to cause an up regulation of adenosine A_{2A} receptors (A2AR) [65] and down regulation of adenosine A_1 receptors (A1R) [66]. The overall increased A2AR and decreased A1R expression caused by iron deficiency results in an excitatory state by increasing glutamate and dopamine release. Therefore, possible alterations in A1R and A2AR ratios in the spinal cord in RLS patients may be the cause of increased glutamate concentrations and mediate a state of spinal hyperexcitability. With regards to the changes in adenosine receptor expression affecting spinal excitability, recently adenosine A1-D2R heteromers have been shown in the mouse lumbar spinal cord [67] which, if downregulated in RLS may lead to spinal hyperexcitability of certain spinal circuits. Moreover, activation of spinal A1R have anti-nociceptive properties, and have been shown to reverse hyperalgesia [68]. The hyperalgesia demonstrated in RLS patients [35–37,40] may develop as a result of the theorised decreased expression of A1R receptors via increased release of glutamate from nociceptive afferents and thus subsequently increasing reflex responses to nociceptive stimuli. Furthermore, adenosine concentrations are known to increase during prolonged wakefulness, producing sleep-inducing effects [69]. Therefore it is possible that increased adenosine concentrations in the evening, combined with the theorised decrease in A1R and increased A2AR in RLS patients, causes an evening increase in glutamate release leading to increased evening spinal reflex excitability in RLS patients [44,45].

Other possible neurochemicals that could be involved in the pathogenesis of RLS are GABA and opioids. However only one study has directly evaluated alterations in GABA concentrations in the brains of RLS patients, finding no differences in GABA concentrations between RLS patients and control participants [63]. With regards to opioids, only two studies have evaluated alterations in the opioid system in RLS patients, both of which focused on the brain [70,71]. One of these studies found RLS severity scores were inversely correlated with post-synaptic opioid receptor binding in some areas serving the medial pain system [70]. The other study proposed that there may be a deficiency in spinal endogenous opioid inhibition of ascending spinothalamic pathways resulting in altered processing at the thalamic level and the feeling of abnormal sensations [71] yet no further studies assessing spinal opioid system changes have been conducted.

Conclusion

In summary, results from studies assessing spinal excitability in RLS indicate that the pathophysiology of RLS probably does not encompass a global increase in spinal excitability but involves complex changes to spinal circuits including interactions between different sensory modalities. Further investigations into spinal neurochemical alterations are required in RLS patients. There may be changes to RLS spinal excitability, both at varying times within RLS patients and between RLS patients and control participants. However, these changes are heterogeneous as not all afferent inputs are affected in the same manner. Therefore this suggests a phase-dependent and modality-dependent state of central sensitisation in RLS patients and, consequently, the theory of global hyperexcitability in RLS patients' needs to be revisited to take into account more subtle features of this syndrome. Future research into the pathophysiology of RLS is important as it will hopefully allow the development of more targeted therapies, including advanced specific drugs to treat the cause of RLS and not just the symptoms.

Practice points

1. RLS symptoms are possibly caused by decreased supraspinal inhibitory influence on the spinal cord and/or an increase in spinal excitability.
2. Combined data from the electrophysiological evaluations of spinal cord activity in RLS patients indicate that the pathogenesis of RLS may involve spinal cord hyperexcitability.
3. Several studies have noted that RLS patients have decreased pain thresholds compared to control participants during both the symptomatic and asymptomatic periods of the disorder. However, RLS patients have hyperalgesia with an absence of allodynia.
4. Spinal reflex analyses in RLS patients have failed to consistently show the expected spinal hyperexcitability through an increase in spinal reflex responses. Only withdrawal reflex responses demonstrate increased excitability in RLS patients compared to healthy controls.
5. A circadian variation in patellar, flexor withdrawal, crossed extensor and plantar reflex responses have been exhibited in RLS patients, with no circadian variation in H-reflex responses.
6. There may be changes to RLS spinal excitability and these may be time of day dependent. However, these changes are heterogeneous as not all afferent inputs appear to be affected in the same manner.

Research Agenda

1. Future electrophysiological studies should attempt to control for confounding variables such as differences in quality of sleep between RLS patients and control participants as well as the possible effects PLMS and increased voluntary evening activity may have on spinal excitability.
2. Additional investigations, with larger samples, are needed to corroborate the findings from current reflex studies in RLS patients as well as more in-depth studies to probe the nature of spinal excitability.
3. Reflex responses should be assessed at multiple times throughout a 24hr cycle in RLS patients to establish whether there is a circadian rhythm in spinal excitability.
4. Future studies should endeavour to assess the sensitivity of afferent nerve fibres in isolation in order to confirm the hypotheses of heterogeneous alterations in afferent nerve fibre sensitivity in RLS patients.
5. Further investigations into the expression of spinal glutamatergic, dopaminergic and adenosinergic receptors as well as spinal glutamate, dopamine and adenosine concentrations are required in RLS patients.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.smrv.2019.05.005.

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