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I_h contributes to increased motoneuron excitability in restless legs syndrome

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Key points

- Restless legs patients complain about sensory and motor symptoms leading to sleep disturbances. Symptoms include painful sensations, an urge to move and involuntary leg movements.
- The responsible mechanisms of restless legs syndrome are still not known, although current studies indicate an increased neuronal network excitability. Reflex studies indicate the involvement of spinal structures. Peripheral mechanisms have not been investigated so far.
- In the present study, we provide evidence of increased hyperpolarization-activated cyclic nucleotide-gated (HCN) channel-mediated inward rectification in motor axons. The excitability of sensory axons was not changed.
- We conclude that, in restless legs syndrome, an increased HCN current in motoneurons may play a pathophysiological role, such that these channels could represent a valuable target for pharmaceutical intervention.

Abstract Restless legs syndrome is a sensorimotor network disorder. So far, the responsible pathophysiological mechanisms are poorly understood. In the present study, we provide evidence that the excitability of peripheral motoneurons contributes to the pathophysiology of restless legs syndrome. *In vivo* excitability studies on motor and sensory axons of the median nerve were performed on patients with idiopathic restless legs syndrome (iRLS) who were not currently on treatment. The iRLS patients had greater accommodation in motor but not sensory axons to long-lasting hyperpolarization compared to age-matched healthy subjects, indicating greater

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inward rectification in iRLS. The most reasonable explanation is that hyperpolarization-activated cyclic nucleotide-gated (HCN) channels open at less hyperpolarized membrane potentials, a view supported by mathematical modelling. The half-activation potential for HCN channels (Bq) was the single best parameter that accounted for the difference between normal controls and iRLS data. A 6 mV depolarization of Bq reduced the discrepancy between the normal control model and the iRLS data by 92.1%. Taken together, our results suggest an increase in the excitability of motor units in iRLS that could enhance the likelihood of leg movements. The abnormal axonal properties are consistent with other findings indicating that the peripheral system is part of the network involved in iRLS.

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Introduction

Restless legs syndrome (RLS) is a sensorimotor disorder with multiple aetiologies. The idiopathic end of the spectrum may be influenced by common allelic variants (Winkelmann *et al.* 2007; Rye, 2015) and is associated with a number of co-morbidities including peripheral neuronal diseases such as polyneuropathy, central disturbances such as stroke and multiple sclerosis, or metabolic disorders such as iron deficiency, anaemia and diabetes (Allen *et al.* 2014; Trenkwalder *et al.* 2016).

Neurophysiological studies suggest that cortical, subcortical and spinal mechanisms intensify sensory input and motor output leading to common positive symptoms, such as the urge to move, uncomfortable leg sensations and periodic leg movements (Trenkwalder & Paulus, 2010; Allen *et al.* 2014; Lanza *et al.* 2017). Transcranial magnetic stimulation (TMS) studies have reported increased excitability within the central nervous system (Lanza *et al.* 2017) and there is considerable evidence that the excitability of spinal circuits is increased in RLS (Bara-Jimenez *et al.* 2000; Aksu & Bara-Jimenez, 2002; Rijsman *et al.* 2005; Scaglione *et al.* 2008; Marconi *et al.* 2012). Clinical observations strengthen the idea of an essential role of the spinal cord in the pathophysiology of RLS: involuntary leg movements in patients with spinal cord injury are identical to sleep-related periodic leg movements seen in RLS (Yokota *et al.* 1991).

The excitability of α-motoneurons depends on intrinsic factors, such as the activity of ion channels and ion pumps. However, these factors are labile: the biophysical properties of α-motoneurons and/or their axons undergo plastic changes after changes in activity (Gardiner *et al.* 2006; Hultborn, 2006), following spinal lesions (Button *et al.* 2008; Boland *et al.* 2009) and in multiple sclerosis (Ng *et al.* 2008). Changes in the excitability of motor axons and presumably the parent neurons occur in pathologies such as diabetes, stroke and porphyria (Horn *et al.* 1996; Jankelowitz *et al.* 2007; Lin *et al.* 2008), all of which are risk factors for developing RLS (Hellmann & Tschudy, 1962; Zobeiri & Shokoohi, 2014; Schlesinger *et al.* 2015). In these excitability studies, the symptoms of RLS were not mentioned, although secondary changes in axonal excitability in diabetes, stroke and porphyria do not necessarily cause RLS symptoms. Thus, the role of motoneuron excitability in restless legs syndrome remains unclear.

To address this unresolved question, we investigated the excitability of motoneurons in patients who had been diagnosed with idiopathic RLS (iRLS) by studying the excitability of motor axons (Bostock *et al.* 1998). Patients with RLS associated with other diseases of the nervous systems were excluded.

Our results suggest greater inward rectification in motor axons in iRLS, and our mathematical modelling is consistent with a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel-driven increase in excitability of motor units in iRLS. This is the first evidence for abnormality in the peripheral system in the sensorimotor network involved in iRLS.

Methods

Ethical approval

Written informed consent was obtained from all patients and healthy subjects. The study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. The procedures were approved by the ethics committee of the University of Göttingen (ethics approval reference number is 20/2/12) and registered by the clinical study management Göttingen.

Patients were included in the study only if they fulfilled the iRLS criteria (Allen *et al.* 2014). Patients suffering from 'secondary' RLS, such as those with clinically relevant polyneuropathy, currently existing anaemia and other neurological or sleep disorders, were not included. No patient or healthy subject was on medication for RLS at the time of the study, and none experienced twitching, cramps or sensory symptoms in the upper and lower limbs during the experimental measurements. Excitability studies were

performed on motor axons in the median nerve in 34 patients (11 men and 23 women) with iRLS and 38 healthy subjects (19 men and 19 women) as normal controls. Similar studies were performed on sensory axons in the median nerve in 14 patients (five men and nine women) and 14 healthy controls (seven men and seven women).

Axonal excitability assessment

Nerve excitability studies of motor axons in the median nerves were carried out via the threshold tracking technique using Qtrac software and the extended TrondNF protocol (UCL Institute of Neurology, London, UK) (Bostock *et al.* 1998). Axonal excitability measurements using the threshold tracking technique are described in detail in several previous studies (Bostock *et al.* 1998; Burke *et al.* 2001; Krarup & Moldovan, 2009). As shown in Fig. 1*A*, stimulation of the median nerve was performed using non-polarizable Ag/AgCl adhesive electrodes (ECG-electrodes; MPC International SA, Luxembourg, Luxembourg). The cathode was placed over the nerve at the wrist and the anode on the radial edge of the forearm (10 cm proximal and away from the median nerve). The compound muscle action potential (CMAP) of abductor pollicis brevis was recorded using surface electrodes connected to an isolated preamplifier (D440; Digitimer Ltd, Welwyn Garden City, UK; gain 200×). The reference electrode was placed on the proximal phalanx of the thumb. A ground electrode was placed on the palm.

Data were digitized at 10 kHz using a data acquisition system (NI USB-6251; National Instruments, Austin, TX, USA). The QtracS software provided stimulus command pulses through the data acquisition system to an isolated linear bipolar constant current stimulator (DS5; Digitimer Ltd). Mains frequency noise was removed with an in-line noise eliminator (Hum Bug 50/60 Hz Noise Eliminator; Quest Scientific, North Vancouver, BC, Canada). Skin temperature was monitored close to the site of stimulation with a thermistor thermometer (EcoScan Temp4 Meter; Eutech Instruments, Singapore) and was kept constant above 32°C throughout the experiment.

The excitability of sensory axons in the median nerve was assessed by stimulation at the wrist as for motor studies. The compound sensory action potential (CSAP) was recorded with ring electrodes around the index finger. The CSAP was measured peak to peak. The test pulses were of 0.5 ms in duration.

Excitability protocols. To investigate the excitability of motor axons, the extended nerve excitability protocol (TrondNF) was used (Tomlinson *et al.* 2010). This includes five stimulation protocols: stimulus–response (SR) curve, strength–duration (SD) properties, threshold electrotonus (TE), current–threshold (*I–V*) relationship and recovery cycle (RC). Excitability studies measure the excitability of a population of axons recruited around the target CMAP/CSAP, which is a fixed fraction of the maximal CMAP/CSAP (40% in the present study). The threshold was defined as the stimulus intensity required to elicit the target CMAP/CSAP (Fig. 1*B*).

SR curve. A SR curve was recorded first using unconditioned stimuli of duration 1.0 ms for motor axons and 0.5 ms for sensory. Stimulus intensity was graded, starting with the maximal CMAP/CSAP and gradually reducing until no CMAP/CSAP could be recorded.

SD properties. To assess the SD properties, motor axons were stimulated with rectangular test pulses with durations of 0.2, 0.4, 0.6, 0.8 and 1.0 ms. For sensory axons, the stimulus durations were 0.1, 0.2, 0.3, 0.4 and 0.5 ms. Strength–duration time constant (SDTC) and rheobase were calculated using Weiss' law (Bostock, 1983; Mogyoros *et al.* 1996).

SDTC was calculated using the formula:

$$\tau_{SD} = t_a\,(I_a - t_b * I_b)\,/\,(I_b - t_a * I_a)$$

where t_a and t_b are the test stimulation durations for the threshold currents I_a and I_b, respectively.

Rheobase (I_{rh}) is the threshold current if the test stimulus could be infinitely long. It was calculated from the same data using the formula:

$$I_{rh} = t * I/(t + \tau_{SD})$$

TE. TE measures accommodation of the threshold stimulus to long subthreshold depolarizing and hyperpolarizing conditioning currents (Burke *et al.* 2001). The standard currents were set as: depolarizing (+20; +40% of the control threshold current, 100 ms long) and hyperpolarizing (–20% and –40% of the control threshold, 100 ms long). To explore inward rectification in greater detail, hyperpolarizing currents, 200 and 300 ms long, were set to –70% and –100% of the control threshold, respectively (Tomlinson *et al.* 2010). Test pulses were applied before, during and after the long-lasting polarizing currents (Fig. 1*C*). TE parameters are termed according to the polarization ('*d*' for depolarizing and '*h*' for hyperpolarizing) and the interval over which the parameter was measured; for example, TE_d 90–100 describes a threshold change produced 90–100 ms after the onset of a depolarizing conditioning current.

***I–V* relationship.** The *I–V* is a measure of rectification and is the threshold analogue of the traditional *I–V* relationship (Burke *et al.* 2001). Motor (or sensory) axons were stimulated with test pulses of duration 1 ms (or 0.5 ms) at the end of 200 ms long polarizing currents

of graded strength [16 strengths, 10% steps from +50% (depolarizing) to –100% (hyperpolarizing) of the control threshold]; increase in excitability was plotted against the strength of the current pulse to produce a threshold '*I–V*' curve (Fig. 1*D*). The steepness of the *I–V* relationship in response to the conditioning hyperpolarizing currents is a measure of inward rectification, whereas the steepness of the curve in the depolarizing direction reflects outward rectification as a result of fast and slow K^+ currents.

Recovery cycle. The recovery cycle measures the excitability following supramaximal stimulation at 18

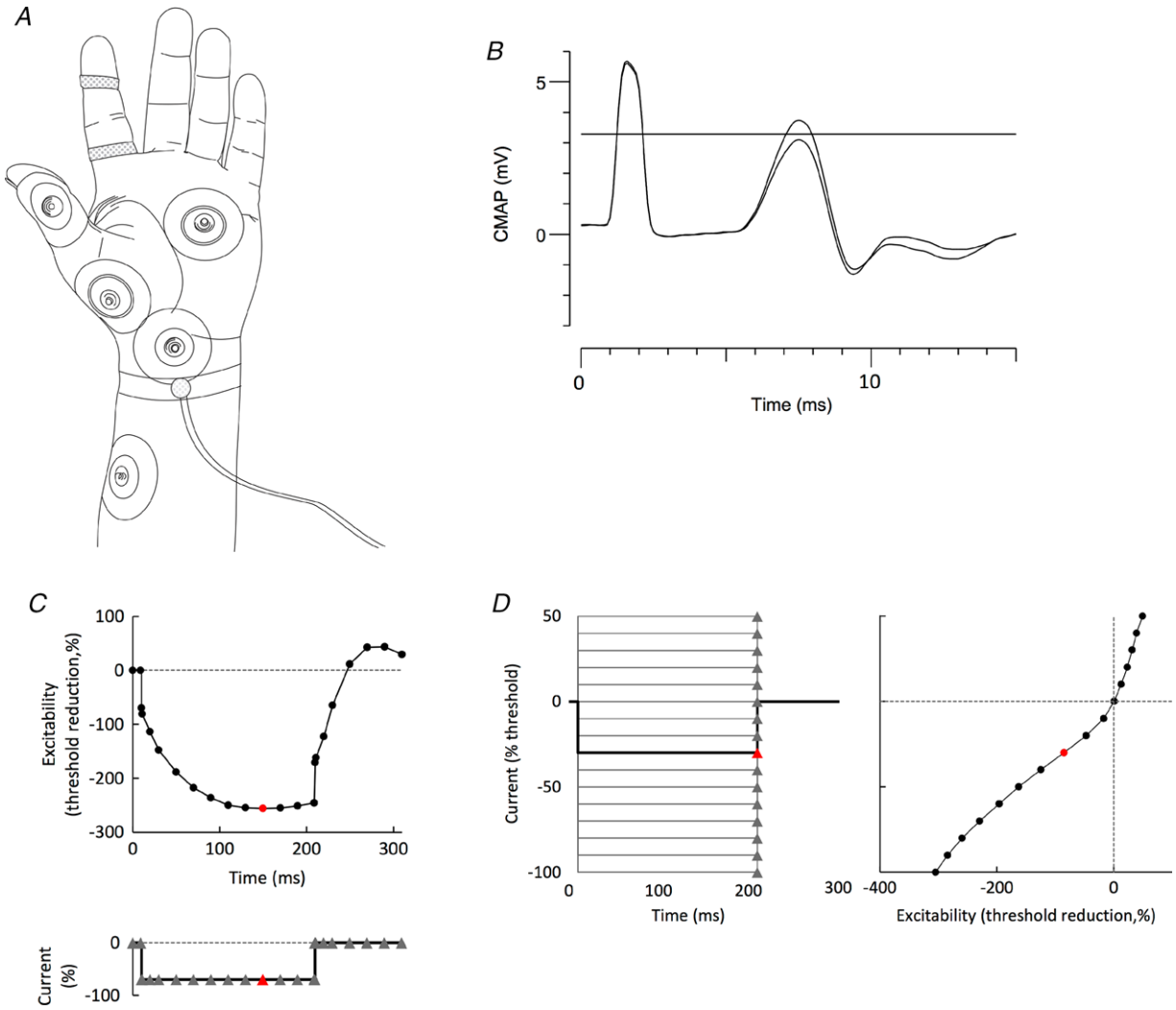

Figure 1. Methodology
A, recording arrangement. Stimuli were delivered at the wrist (cathode) using Ag/AgCl electrodes with the anode 10 cm distal on the radial edge of the forearm. CMAPs were recorded over the thenar eminence, with the reference electrode on the proximal phalanx of the thumb. CSAPs were recorded using disposable Ag/AgCl ring electrodes on digit 2. The same ground (in the palm) was used for both motor and sensory recordings. A thermistor was strapped around the wrist to record skin temperature close to the site of stimulation (Howells *et al.* 2013). *B*, principle of threshold tracking. A target threshold was set, as indicated by the horizontal line, here ~3 mV, which is ~40% of the size of the maximal CMAP (or CSAP). If the target response exceeded that threshold (e.g. as demonstrated by the larger CMAP), the intensity of the next test stimulus was reduced. If the target response was less than the threshold value (as in the smaller CMAP), the intensity of the next test stimulus was increased. *C* and *D*, application of the technique to the recording of TE to a –70% hyperpolarizing current (*C*) and to the *I–V* relationship (*D*). *C*, threshold was measured at the intervals indicated by triangular markers, before, during and after a square wave hyperpolarizing current lasting 200 ms, set to –70% of the control threshold. *D*, threshold was measured 200 ms after the onset of 15 square-wave polarizing currents from +50% (depolarizing, top left) to –100% (hyperpolarizing, bottom). The change in excitability in the right panel is equivalent to a conventional *I–V* relationship. In (*C*) and (*D*), the measurements on the threshold plots in red are indicated on the current plots in red.

conditioning test intervals, in a roughly logarithmic sequence from 2 to 200 ms following this supramaximal stimulus. To ensure accurate measurements at short conditioning test intervals, the CMAP (CSAP) produced by the conditioning stimulus alone was subtracted online from the combined response to the conditioning and test stimuli.

Statistical analysis

Group data are reported as the mean ± SD. Individual data were tested for normality using the Kolmogorov–Smirnov test. The mean values for the two groups (normal and iRLS) were compared using Student's *t* tests for unpaired data. Measures that follow a log-normal distribution were calculated using the geometric mean and geometric SD. Multiple comparisons were corrected using the Holm–Bonferroni method (Holm, 1979).

Mathematical modelling

To explore the mechanisms responsible for increased inward rectification seen in the iRLS recordings, the group data were modelled using the 'Bostock' mathematical model of a human motor axon (Bostock *et al.* 1991), as updated by Howells *et al.* (2012). This contains both nodal and internodal compartments with voltage-gated ion channels, ion pumps and leak conductances that characterize the excitability of human myelinated axons. The discrepancy between modelled and recorded data was minimized using an iterative method. The overall discrepancy was defined as the weighted sum of the Euclidean distances (or square root of the sum of the squares) between the model and group data. The weights for each of the four measures were: SD time constant = 0.5; TE = 3; RC = 1; *I–V* relationship = 2.

Results

Excitability of motor axons in the median nerve

In total, 72 individuals were studied, 34 patients (11 men and 23 women) with iRLS and 38 healthy volunteers (19 men and 19 women) as a normal control group. None of the iRLS patients had upper limb motor or upper limb sensory symptoms. The ages of the iRLS and control groups were similar: 51 ± 16.3 years and 47.6 ± 12.8 years, respectively ($P = 0.27$).

All participants underwent excitability studies on the median nerve at the wrist, and no significant discomfort was reported by the subjects. The data conformed to a normal distribution (or did so after log transformation) and the results are shown in Figs 2–4.

There were no significant differences between the iRLS patients and controls in the maximal CMAP amplitudes ($P = 0.1$) (Fig. 2*A*) and in threshold-related measures: stimulus for a half-maximal response ($P = 0.9$) (Fig. 2*C*) and SR curves (SR slope, $P = 0.35$) (Fig. 2*B*), SDTC ($P = 0.17$) (Fig. 3*A*) and rheobase ($P = 0.93$) (Fig. 3*B*).

There were no significant differences between the iRLS and control groups in measures of the recovery of excitability following a supramaximal conditioning stimulus (relative refractory period, $P = 0.89$, extent of refractoriness, $P = 0.6$; superexcitability, $P = 0.73$; late subexcitability, $P = 0.68$) (Fig. 2*F* and 3*C*). The measures shown in Figs 2*A–F* and 3*A–C* are those most sensitive to small changes in resting membrane potential (Kiernan

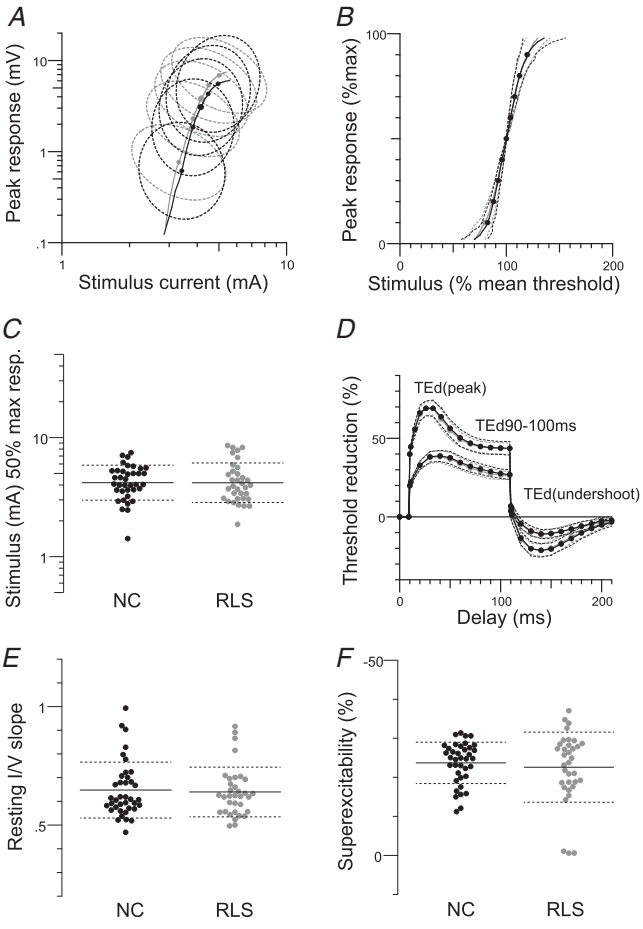

Figure 2. Evidence against a change in resting membrane potential
Excitability data for motor nerves in normal controls (NC) ($n = 38$) and RLS patients ($n = 34$). *A* and *B*, maximal CMAPs ($P = 0.1$), black: normal controls, grey: iRLS; dotted circles (*A*) and lines (*B*) indicate 1 SD). *C*, stimulus for a half-maximal response ($P = 0.9$), black: normal controls, grey: iRLS; dotted lines indicate SD. *D*, mean data for depolarizing TE (note that the patient data and the control data superimpose perfectly such that it is difficult to identify separate data points). TE_d (peak) ($P = 0.8$); TE_d 90–100 ms ($P = 0.86$); TE_d (undershoot) ($P = 0.87$). *E*, resting *I–V* slope ($P = 0.76$). *F*, superexcitability ($P = 0.6$).

& Bostock, 2000) and these findings suggest that there was little difference in resting membrane potential in the patients and control subjects.

In TE, the accommodation to hyperpolarization was greater in the patient group, and this difference was greater the stronger the hyperpolarization (Fig. 4). This is as would be expected with greater I_h. Accordingly, the increase in threshold to hyperpolarizing currents was smaller in iRLS with conditioning currents of –70% and –100%, as shown in Fig. 4*A* (for –70%: iRLS, –237.4 ± 29.2%; controls, –260.8 ± 34%; $P = 0.0027$, Holm–Bonferroni adjusted $P = 0.011$; for –100% iRLS, –354.5 ± 35%; controls, –385.3 ± 42.2%; $P = 0.0014$, adjusted $P = 0.0072$). The patients with iRLS had greater minimum *I–V* slope (iRLS, 0.27 ± 0.05; controls, 0.25 ± 0.04; $P = 0.011$, adjusted $P = 0.034$) (Fig. 4*C*). TE_h (90–100 ms) and TE_h (slope 101–140 ms) were also significantly different (iRLS, –114.3 ± 15.2%; controls, –122.5 ± 18.7%; $P = 0.0416$, adjusted $P = 0.0416$; iRLS, 2.04 ± 0.34; controls, 2.28 ± 0.49; $P = 0.018$, adjusted $P = 0.036$) (Fig. 4*A* and *C*). The resting *I–V* slope was not significantly different between iRLS and the control group (iRLS, 0.63 ± 0.1; controls, 0.65 ± 0.1; $P = 0.75$) (Figs 2*E* and 4*C*).

Mathematical modelling

The differences in the axonal excitability of the median nerve were modelled using the modified 'Bostock'

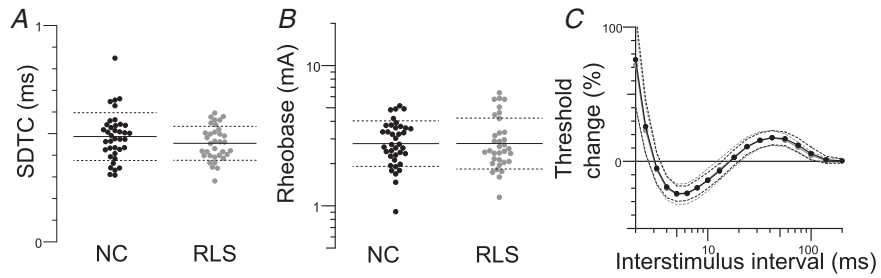

Figure 3. SD properties and recovery cycles
Excitability data for motor nerves in normal controls ($n = 38$; black lines) and RLS patients ($n = 34$; grey lines). Dotted lines indicate the SD. SD time constant ($P = 0.09$) (*A*) and rheobase ($P = 0.82$) (*B*) were not different. *C*, no significant differences in measures of the recovery cycle (relative refractory period, $P = 0.87$; extent of refractoriness, $P = 0.6$; superexcitability, $P = 0.6$; late subexcitability, $P = 0.53$).

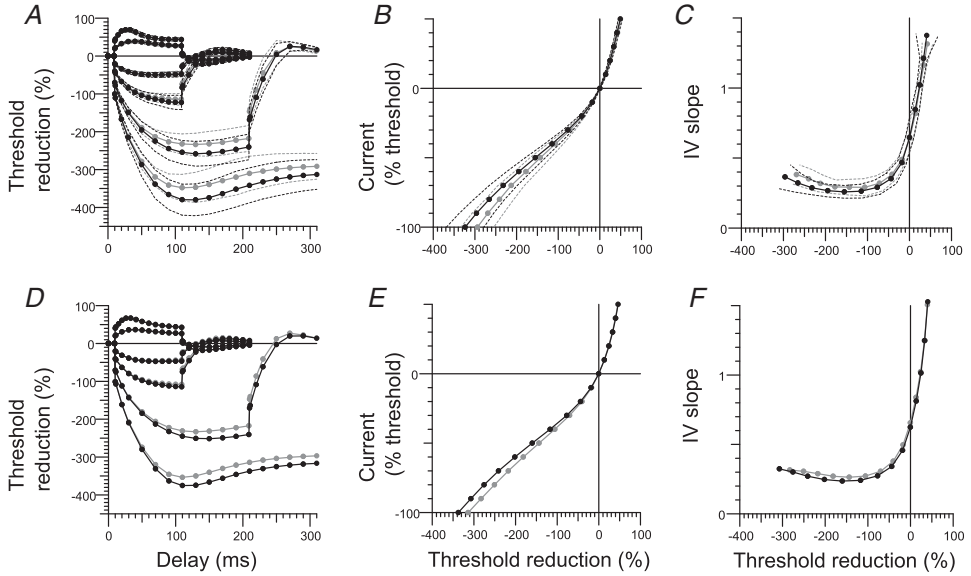

Figure 4. Evidence for greater I_h in median nerve with the best-fit from the mathematical model
Extended excitability data for motor axons in the median nerve. *A*, *B* and *C*, upper row: group data (mean and SD); normal controls ($n = 38$; black filled circles) and RLS patients ($n = 34$; grey filled circles) and best-fit from the Bostock motor axon model (*D*, *E* and *F*; lower row: black circles indicate the model in control subjects, and the modelled changes in RLS patients are shown as grey circles). *A* and *D*, TE for conditioning levels of +20%, –20%, +40%, –40%, –70% and –100% of control threshold. *B* and *E*, *I–V* for 200-ms conditioning stimuli. *C* and *F*, *I–V* slope (threshold conductance): resting *I–V* slope (*r*): calculated from currents between –10% and +10%, shown at $x = 0$; minimal *I–V* slope.

model of a human motor axon. The only significant changes in axonal excitability appeared when the nerve was significantly hyperpolarized (Fig. 4*A*–*C*: measured data, Fig 4*D*–*F*: modelled data), indicative of a change in the hyperpolarization-activated current, I_h. All of the parameters associated with I_h were examined (Fig 4*D*–*F*) The most reasonable explanation is that HCN channels open with a lesser degree of hyperpolarization. Accordingly, the half-activation potential for HCN channels (B_q) was the single best parameter that accounted for the difference between normal controls and iRLS data. A 6 mV depolarization of B_q reduced the discrepancy between the normal control model and the iRLS data by 92.1%.

Excitability of sensory axons in the median nerve

Fourteen patients with iRLS and 14 healthy controls were recruited. Based on the motor findings, these samples should have been adequately powered to detect identical changes in I_h: indeed, samples of only five subjects would have a power of 81.7% to detect the change seen in motor axons at the 5% level in a one-tailed *t* test (one-tailed because the direction of change was known). However, because the properties of sensory and motor axons differ, larger samples were studied. There were no significant changes in the excitability of sensory axons in the median nerve (Fig. 5, round symbols).

Discussion

In the present study, we report data showing for the first time that the intrinsic biophysical properties of peripheral motor axons are altered in patients suffering from iRLS. Excitability studies in patients with a clinical diagnosis of iRLS not on medication have increased inward rectification in motor axons of the median nerve. Mathematical modelling supports this interpretation and suggests that the responsible mechanism is a depolarizing shift in the voltage dependence of HCN channels.

HCN activity: the 'chicken and egg' issue

HCN channels are known to be 'plastic'. One major factor that regulates channel function is neuronal activity. The simplest explanation for the greater inward rectification is that it is driven by both 'restless' involuntary (in the context of periodic leg movements) and voluntary (in the context of the urge to move) motor activity. In many respects, this explanation complements that for patients who had suffered a stroke and in whom evidence for less HCN activity was reported (Jankelowitz *et al.* 2007). In addition, peripheral hypoxia, which is known to increase HCN current, has been demonstrated in RLS (Salminen *et al.* 2014), although this factor is probably not important for median motor axons remote from the 'restless' activity.

However, the evidence for greater inward rectification was found for median nerve axons, and none of the patients had relevant upper limb symptoms. This raises the possibility that greater HCN current may be a primary and generalized phenomenon, rather than secondary to focal heightened 'restless' contractions or peripheral hypoxia. In this regard, the threshold tracking technique has proven to be sufficiently sensitive to identify biophysical abnormalities in motor axons, even in asymptomatic motor nerves in various neurological diseases (Jankelowitz *et al.* 2007; Ng *et al.* 2008; Tomlinson *et al.* 2012). Moreover, a generalized disorder could also explain why the upper limbs are often symptomatic (in 21–57% of cases) (Allen *et al.* 2014) or become so when iRLS patients are started on high dosages of dopaminergic drugs (Allen & Earley, 1996), comprising a previously unexplained phenomenon. Further studies on iRLS patients during such treatment could clarify this issue.

In earlier studies on healthy subjects, we have demonstrated that, although the between-subject variability of I_h is high, within-subject variability is quite

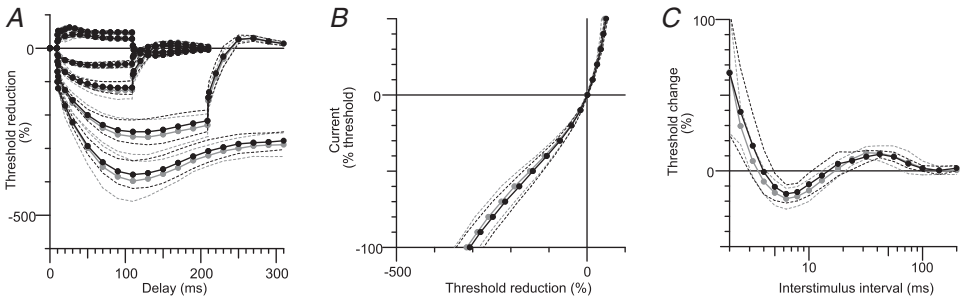

Figure 5. No changes in axonal excitability in sensory axons of the median nerve in RLS
Extended excitability data. *A*, *B* and *C*, normal controls ($n = 14$; black filled circles) and RLS patients ($n = 14$; grey filled circles) for sensory axons (median nerve) (mean ± SD). *A*, TE for conditioning levels of +20%, –20%, +40%, –40%, –70% and –100% of control threshold. *B*, *I–V* relationship for 200 ms conditioning stimuli. *C*, recovery cycle.

low, even in test–retest situations (Tomlinson *et al.* 2010; Howells *et al.* 2013), and also that the between-subject variability can be explained by differences in the voltage for half-activation of HCN channels (Howells *et al.* 2012). Different subjects have different I_h activity levels, presumably related to life-style factors, to which I_h is exquisitely sensitive (Pape, 1996; Robinson & Siegelbaum, 2003; Wahl-Schott & Biel, 2009), and possibly genetic factors, which are prominent in idiopathic RLS (Rye, 2015; Winkelmann *et al.* 2007; Trenkwalder *et al.* 2016). In this respect, RLS patients may merely be at one end of a 'normal' spectrum of I_h activity. It is relevant that plastic changes in the activity of I_h occur largely through changes in channel gating (Biel *et al.* 2009; Wahl-Schott & Biel, 2009), much as suggested by modelling in the present study and also prevously by Howells *et al.* (2012).

Functional consequences of greater HCN current

We presume that the greater I_h on motor axons reflects greater I_h in the parent motoneurons. HCN current is depolarizing and non-inactivating and, accordingly, would increase the probability of motoneuron discharge. There was no significant change in resting membrane potential in motor axons, although the axonal change probably does not quantitatively replicates the change in the parent motoneurons. In addition, a depolarizing shift in membrane potential could have been readily offset by small changes in multiple properties, which are changes that cannot be identified in the modelling. The changes in the experimental data were not large, although this does not mean that the biological effect of these changes was small, particularly at the motoneuron level.

The kinetics of HCN channels are in the range of hundreds of milliseconds to seconds (Wahl-Schott & Biel, 2009) and this is too slow for the channels to play a pacemaker role in the discharge of individual motoneurons. However, HCN channels might well be suited to modulate the bursting nature of the motoneurons in increased motor activity as a result of the urge to move. It is reasonable to postulate that, in the presence of increased I_h, any input to the motoneuron, peripheral or descending (Fig. 6) would have a greater probability of facilitating motoneuron discharge, such that natural drives reaching the axon hillock might then trigger restless activity.

RLS is a complex sensorimotor network disorder

Axonal excitability studies document excitability at the site of stimulation: the peripheral axon. Thus, our data show for the first time that hyperexcitability in iRLS can also be found in peripheral motor axons. It is therefore important that the findings using other neurophysiological techniques be (re)-interpreted considering the whole network proposed for RLS, including the peripheral nervous system (Fig. 6).

Neurophysiological, neuroimaging and neuromodulatory studies indicate the increased excitability of a neuronal network encompassing cortical, subcortical and spinal areas (Lanza *et al.* 2017) in both idiopathic and secondary RLS (Trenkwalder *et al.* 2016). From a neurophysiological point of view, most techniques identify alterations in targeted structures; for example, cortical excitability in TMS studies (Magalhaes *et al.* 2015; Lanza *et al.* 2017) and spinal excitability in reflex studies (e.g. diminished inhibitory mechanisms: Rijsman

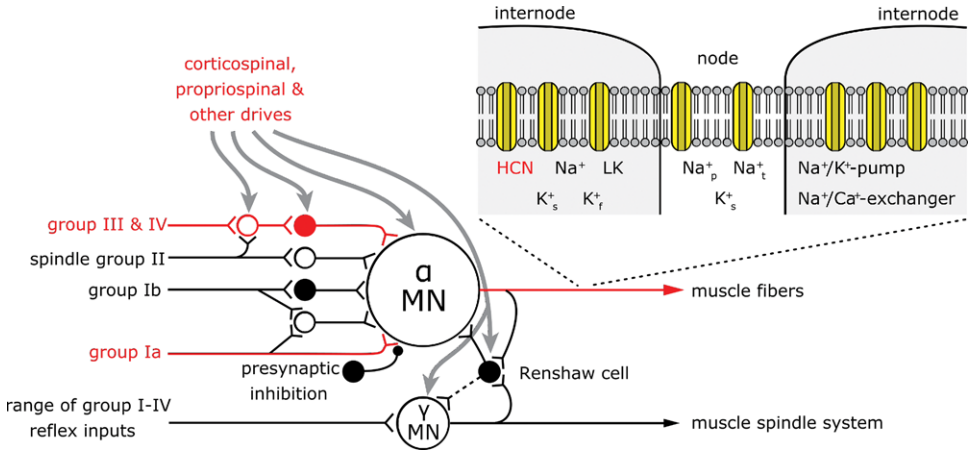

Figure 6. Restless legs syndrome is a network disorder
Motoneurons receive various central and peripheral inputs. It has already been shown that in RLS input to the motoneuron is increased via group IV, group I afferents and central efferents (red coloured input; Bara-Jimenez *et al.* 2000; Rijsman *et al.* 2005; Lanza *et al.* 2017). Our data show for the first time that hyperexcitability in iRLS can also be found in peripheral motor axons. Na^+_p: persistent sodium channel; Na^+_t: transient sodium channel; K^+_f: fast potassium channel; K^+_s: slow potassium channel (Adapted from Gandevia, 2001).

et al. 2005; Scaglione *et al.* 2008; Marconi *et al.* 2012; as well as enhanced and state-dependent flexor reflex: Bara-Jiminez *et al.* 2000; Aksu & Bara-Jiminez, 2002). QST (Bachmann *et al.* 2010) and cutaneous silent period studies (Isak *et al.* 2011) involve both peripheral nerve and spinal excitability. On their own, these studies do not control for a contribution from non-investigated areas. For example, TMS studies need peripheral reflex studies to exclude additional spinal influences.

The excitability of spinal motoneurons depends on intrinsic factors and on the descending and peripheral influences acting upon them. The enhanced excitability seen in TMS and reflex studies could therefore have resulted from a primary change elsewhere in the neuraxis. However, the greater inward rectification in motor axons in the present study presumably reflects increased I_h in motoneurons, implying a difference in the intrinsic properties of those motoneurons. We cannot exclude the fact that this change in motoneuron properties was an adaptation to a change in descending or peripheral inputs but, if this was the case, an 'indelible mark' would have been left on the motoneurons, altering their responsiveness.

Measurements on sensory axons did not show changes in excitability. This suggests that increased inward rectification in RLS is specific to motor axons and is not a generalized (patient-specific) phenomenon. It should be noted that the threshold-tracking techniques used in the present study document the biophysical properties only of large myelinated fibres, and not those of small myelinated and unmyelinated fibres, which are probably involved in RLS (Bachmann *et al.* 2010). Thus, future studies could be focussed on the biophysical properties of small fibres in RLS.

Clinical implications

The findings of the present study are similar to those of our previous study of benign cramp fasciculation syndrome (Czesnik *et al.* 2015). This is also a condition with increased muscle activity and it is tempting to suggest that similar mechanisms are responsible for the increased I_h.

I_h has also been implicated in neuropathic pain (Chaplan *et al.* 2003; Momin *et al.* 2008; Biel *et al.* 2009). Again there is an analogy to the situation with RLS in which patients complain of an urge to move the legs, usually associated with unpleasant leg sensations, often reported as pain or discomfort (Trenkwalder & Paulus, 2010). A common feature of all these conditions is hyperexcitability of the relevant neuronal networks. I_h is a depolarizing current and greater I_h in this network would contribute to or accentuate any hyperexcitability.

Currently, dopaminergic agents are recommended for RLS (Trenkwalder *et al.* 2015). The present findings suggest that modulators of I_h might be beneficial when safe medications become freely available.

Taken together, we conclude that an increase in motoneuronal I_h as suggested by the present axonal excitability studies plays an important role in fine-tuning the final common path not only under physiological circumstances, but also in a variety of different clinical conditions.

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Additional information

Competing interests

The authors declare that they have no competing interests.

Author contributions

The experiments were performed in the Department of Clinical Neurophysiology, Medical School Göttingen.

DC, AW, CT, DB and WP were responsible for the conception and design of work. DC, MB, EV and OK were responsible for the acquisition of data. DC and JH were responsible for the analysis of data. DC, JH, DB and WP were responsible for the interpretation of data. DC, MB, EV, OK, DB and WP were responsible for drafting the paper. DC, JH, AW, CT, DB and WP were responsible for critically revising the paper for important intellectual content. All authors read and approved the final version of the manuscript submitted for publication.

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